

# nutritional factors involved in the goitrogenic action of cassava



Editors: F. Delange, F.B. Iteke, and A.M. Ermans



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# **Nutritional Factors Involved in the Goitrogenic Action of Cassava**

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## Contents

Foreword	J. Gill	5
Préface	F.B. Iteke	7
Contributors		9
Acknowledgments		11
Introduction	A.M. Ermans and F. Delange	13
Introduction	A.M. Ermans et F. Delange	17
<i>Chapter 1</i>	Biochemical and statistical methods P. Bourdoux, G. Putzeys, R. Lagasse, and A. Van Steirteghem	20
<i>Chapter 2</i>	Epidemiology of goitre and malnutrition and dietary supplies of iodine, thiocyanate, and proteins in Bas Zaire, Kivu, and Ubangi P. Hennart, P. Bourdoux, R. Lagasse, C. Thilly, G. Putzeys, P. Courtois, H.L. Vis, Y. Yunga, P. Seghers, and F. Delange	25
<i>Chapter 3</i>	Influence of the dietary balance of iodine/thiocyanate and protein on thyroid function in adults and young infants R. Lagasse, P. Bourdoux, P. Courtois, P. Hennart, G. Putzeys, C. Thilly, M. Mafuta, Y. Yunga, A.M. Ermans, and F. Delange	34
<i>Chapter 4</i>	Influence of dietary goitrogens during pregnancy in humans on thyroid function of the newborn F. Delange, C. Thilly, P. Bourdoux, P. Hennart, P. Courtois, and A.M. Ermans	40
<i>Chapter 5</i>	Cassava products: HCN content and detoxification processes P. Bourdoux, P. Seghers, M. Mafuta, J. Vanderpas, M. Vanderpas-Rivera, F. Delange, and A.M. Ermans	51
<i>Chapter 6</i>	Breast feeding, thiocyanate metabolism, and thyroid function in young infants in severe endemic goitre J. Vanderpas, M. Vanderpas-Rivera, P. Bourdoux, M. Dramaix, R. Lagasse, P. Seghers, F. Delange, A.M. Ermans, and C. Thilly	59
<i>Chapter 7</i>	Role of the balance between the dietary supplies of iodine and thiocyanate in the etiology of endemic goitre in the Ubangi area P. Courtois, P. Bourdoux, R. Lagasse, A.M. Ermans, and F. Delange	65
<i>Chapter 8</i>	Serum levels of free amino acids in mothers at delivery, newborns, and adult males H.L. Vis, A. Vuye, and P. Hennart	70

<i>Chapter 9</i>	Experimental study of mechanisms responsible for mental retardation resulting from cassava ingestion	<b>E. Colinet, A.M. Ermans, and F. Delange</b>	74
<i>Chapter 10</i>	Summary and general conclusions	<b>F. Delange, P. Bourdoux, and A.M. Ermans</b>	84
<i>Chapitre 10</i>	Résumé et conclusions générales	<b>F. Delange, P. Bourdoux et A.M. Ermans</b>	87
References			90

## Foreword

This monograph reports on the final phase of investigations completed in Zaire and is a follow-up to the first publication, which dealt with the role of cassava (*Manihot esculenta* Crantz) in the etiology of endemic goitre and cretinism.

The two main objectives of the second phase of research were: to study the nutritional conditions required in humans for cassava to induce endemic goitre and cretinism, with special attention to the relative roles of iodine and proteocaloric intake in the presence of a cassava-based diet; and to study the mechanisms responsible for mental retardation resulting from a cassava-based diet and, more specifically, to define the role of thyroid failure in early life and the direct toxic effect of cyanide on the central nervous system.

The results confirm that the development of goitre in the presence of a cassava-based diet depends upon the balance between dietary supplies of iodine (I) and thiocyanate (SCN). A key finding of this study is the establishment of critical threshold values of the I/SCN ratio related to normal conditions, endemic goitre, and hyperendemic cretinism. Factors that contribute to the I/SCN ratio, as well as the methodology for estimating it in field studies, have also been elaborated. In addition, it has been shown that, in the presence of severe protein calorie malnutrition, the prevalence of goitre may not be a valid index of cassava toxicity and its effect on thyroid function. It is important that both the level of iodine and thiocyanate supplies be determined in areas of endemic goitre.

The studies stress the particular vulnerability of newborns and pregnant women, as well as infants and children, to cassava toxicity. To minimize the risk of mental retardation, newborns in high-risk populations should be screened for congenital hypothyroidism. Appropriate prophylaxis and treatment of pregnant women and newborns, as well as the promotion of breastfeeding, can play a role in preventing the development of endemic cretinism.

The findings presented here make fascinating reading and go a long way to indicate the relationship between cassava intake and thyroid conditions in humans. They also emphasize the need for further agricultural research to develop varieties of cassava that contain minimal amounts of linamarin, and for studies on effective detoxification techniques at the village level.

The results of this 7-year study have important public health implications for areas where cassava is consumed as a staple food. The Zairian and Belgian scientists involved in this work are to be congratulated for their achievements under extremely difficult field conditions.

**J. Gill, MD**  
**Director**  
**Health Sciences Division**  
**IDRC**

## Préface

L'importance de la recherche scientifique en tant que facteur de développement n'est plus à démontrer. Le Zaïre, tout comme plusieurs pays en développement, se heurte de plus en plus aux problèmes cruciaux du développement économique et social.

La solution de ces problèmes exige qu'on ne s'y prenne pas de manière empirique et désordonnée. C'est pourquoi, en 1975, le pouvoir public zaïrois a réorganisé le secteur de recherche en créant une institution publique nationale dénommée "Institut de recherche scientifique" (IRS).

Cette institution est aujourd'hui un instrument du développement à qui l'Etat assigne expressément la mission d'effectuer, promouvoir et coordonner la recherche dans les domaines de la science, de la technique et de l'industrie sur toute l'étendue de la République.

Dans son plan d'action, l'IRS donne une importance toujours grandissante aux problèmes de la santé publique et des recherches médicales.

Au cours de la décennie qui s'achève, la situation sanitaire est restée des plus préoccupantes dans le monde en général et dans les pays en développement en particulier. Dans ces derniers pays, les statistiques de l'OMS signalent une recrudescence d'un certain nombre de maladies et autres grandes endémies. Cette situation aurait pour origine la pauvreté, les infections, la malnutrition, l'absence d'eau potable, les dangers inhérents à l'environnement naturel, social, et alimentaire etc.

En vue d'améliorer la santé des populations avant la fin du siècle, l'OMS insiste sur la nécessité d'un effort mondial concerté. L'OMS invite les organisations des Nations Unies, les organisations intergouvernementales et surtout les gouvernements des pays en développement à élaborer des politiques et des programmes sanitaires appropriés à tous les échelons : national, régional et interrégional. Elle recommande également la promotion et le développement de la recherche bio-médicale ainsi que la réorganisation efficace des services de santé. Dans ces programmes de recherche, l'OMS insiste sur la priorité à accorder aux recherches visant l'amélioration de l'état nutritionnel des populations et l'amélioration qualitative des aliments, car la malnutrition est cause majeure de mauvaise santé et de mortalité.

Aussi, l'étude scientifique des maladies endémiques telles que le goitre et le crétinisme occupe-t-elle une place de choix dans les objectifs de l'IRS du fait du nombre trop élevé des cas enregistrés dans trois régions du pays : l'Equateur, le Haut-Zaïre et le Kivu.

Le goitre et sa complication majeure, à savoir le crétinisme endémique, constituent, dans ces parties du territoire zaïrois, un véritable fléau. La prévention de ces endémies suscite de la part du pouvoir public une attention toujours croissante, afin de préserver de ces maladies la main-d'oeuvre nécessaire à l'effort du développement.

Par ailleurs, la lutte contre ces maladies endémiques comme beaucoup d'autres maladies tropicales, n'est pas sans passé au Zaïre. Cette histoire remonte à la fin du XIX<sup>e</sup> siècle avec les essais d'établissement, sur l'initiative du roi Léopold II en 1892, des "Laboratoires médicaux pour l'étude scientifique des conditions pathologiques au Congo" et elle s'installera définitivement sur sa trajectoire actuelle avec la création, en 1947, de l'Institut de recherche scientifique en Afrique centrale (IRSAC) qui est devenu aujourd'hui l'un des centres importants de l'IRS.

Déjà entre 1965 et 1972, l'huile iodée (Lipiodol ultra-fluide) qui est actuellement utilisée dans le programme d'éradication du goitre et du crétinisme endémiques par

le centre de l'IRS Gemena, a été utilisée la première fois au Zaïre pour traiter un grand nombre de personnes de l'île Idjwi au Kivu.

En 1972, les résultats encourageants obtenus à l'île Idjwi ont permis non seulement de poursuivre le travail entrepris au Kivu mais aussi d'étendre ces activités vers une endémie goitreuse d'une extrême gravité dans le nord de l'Equateur et le Haut-Zaïre.

En 1972 et 1973, grâce à plusieurs missions effectuées par le docteur Thilly dans l'Ubangi-Mongala en Equateur, l'existence d'une endémie goitreuse qui était mal identifiée a été précisée. Une "section goitre" distincte de celle de Kivu, placée sous la responsabilité du professeur Ermans fut alors créée à Gemena.

Depuis 1975, la section du goitre CEMUBAC poursuit la collaboration fructueuse avec l'IRS sous le nom de "Programme National d'Eradication du Goitre au Zaïre".

L'un des sujets de recherche abordés en 1975 fut l'appréciation du mode d'action du manioc (action au niveau de la pompe à iodure thyroïdienne ou au niveau de l'organification intrathyroïdienne de l'iodure).

Un sujet semblable fut financé par le Centre de recherches pour le développement international (CRDI) du Canada en 1979. Il s'agit du projet "Facteurs nutritionnels et d'environnements dans la toxicité du manioc chez l'homme".

Cette étude a pu démontrer que dans l'endémie de l'Ubangi, comme dans celle du Kivu, la carence en iode ne constitue pas le seul facteur étiologique du goitre endémique et que la consommation du manioc non roui constitue un facteur précipitant. Dans le nord-ouest du Zaïre, le développement du goitre endémique est donc lié de manière critique à la balance entre les apports en iode et en thiocyanate par l'alimentation.

En 1980, le CRDI avait publié une première monographie intitulée "Role of cassava in the etiology of endemic goitre and cretinism". Cette monographie résumait les travaux effectués en collaboration par l'IRS Gemena et la section goitre du CEMUBAC dans le domaine du goitre endémique au Zaïre et était essentiellement centrée sur la démonstration du rôle toxique joué par le manioc sur la fonction thyroïdienne chez l'homme. Les éditeurs de cette première publication étaient A.M. Ermans, F. Delange, R. Ahluwalia et le président du Conseil scientifique de l'IRS, le professeur Mbulamoko, qui avait fait une préface pour cette première monographie.

Le CRDI dans un deuxième temps, avait financé une nouvelle étude dont le but essentiel était de préciser les conditions nutritionnelles requises pour que le manioc exerce une action toxique sur la thyroïde. Cette deuxième étude a consisté en une comparaison des conditions épidémiologiques, nutritionnelles et métaboliques existantes dans l'Ubangi (Gemena), le Bas-Zaïre (Kisantu) et le Kivu (Kabare). C'est pour cette deuxième monographie que l'honneur m'échoit de rédiger la présente préface.

Ce vaste et important programme de recherche a été rendu possible grâce à l'apport financier et à l'assistance inestimable des organismes tant zaïrois, belges, qu'internationaux. Il s'agit du Conseil exécutif du Zaïre (IRS et Département de la Santé), de la Section goitre du CEMUBAC, du CRDI et du Corps de la Paix Américain.

Je tiens à féliciter ici le CEMUBAC et le CRDI qui ont initié le projet et concouru à la constitution de l'infrastructure appropriée de recherche ainsi que l'équipe dynamique des chercheurs qui ont participé à la réalisation et à la publication de cette monographie.

Enfin, j'espère que cette publication apportera une nouvelle contribution à l'éradication du goitre et du crétinisme au Zaïre. Mon vœu le plus ardent est de voir l'Institut de recherche scientifique du Zaïre et la section goitre du CEMUBAC continuer à entretenir comme dans le passé d'excellentes relations de collaboration scientifique toujours fructueuses.

**Professeur Iteke Fefe Bochoa**

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## Introduction

A.M. ERMANS AND F. DELANGE

Previous studies in Zaire have shown that cassava has a definite antithyroid action in humans and animals resulting in the development of endemic goitre and cretinism (Ermans et al. 1980a). This action is due to the endogenous release of thiocyanate (SCN) from linamarin, a cyanogenic glucoside contained in cassava, in particular in the tuberous roots (Bourdoux et al. 1980a).

However, despite the fact that cassava is consumed on a large scale in the tropics (Nestel 1973; Phillips 1974), goitre and cretinism are not found in all populations whose staple food is cassava (Kelly and Snedden 1960; Kochupillai et al. 1980; Medeiros-Neto and Dunn 1980).

One possible explanation for the lack of goitrogenic action of cassava in some populations may be that they have a high iodine intake. In fact, in the areas investigated in Zaire (Idjwi Island, Kivu and Ubangi, Equateur), the antithyroid action of cassava was observed in the presence of a severe iodine deficiency (Delange et al. 1968; Ermans et al. 1969, 1980a; Thilly et al. 1972; Delange 1974). Moreover, the correction of iodine deficiency by injections of slowly resorbable iodized oil resulted in the eradication of endemic goitre and cretinism and in a normalization of thyroid function (Thilly et al. 1973; Thilly 1979; Lagasse et al. 1980b). This

last observation agrees with experimental findings in rats indicating that the goitrogenic effect induced by large doses of SCN is completely suppressed by iodine supplements (Ermans et al. 1980b).

Studies conducted in Sicily proved that SCN may have a goitrogenic action even when the iodine supply is not as dramatically reduced as that observed in Zaire (Delange et al. 1978).

The question, therefore, is "at what level of iodine intake does ingestion of SCN precursors, such as those found in cassava, alter the iodine metabolism of the thyroid gland and play a definite role in the etiology of endemic goitre?"

Another nutritional factor that may be involved in the goitrogenic action of cassava in humans is the protein calorie intake since the endogenous conversion of cyanide (HCN) into SCN requires sulfur amino acids (Ermans et al. 1972; Oke 1973; Osuntokun 1973; Barrett et al. 1978; Bourdoux et al. 1980a). Experimentally in pigs, protein deficiency protects against the antithyroid action of cassava by reducing the quantity of SCN arising from HCN (Tewe 1976). It has also been shown experimentally that the presence of protein calorie deficiency impairs the development of goitre due to a goitrogenic diet (Aschkenazy et al. 1962; Cowan and Margossian 1966; Shrader et al. 1977).

The observation in the Ubangi endemic goitre area of retarded psychomotor development in young infants who do not show the other features of endemic cretinism (Lagasse et al. 1980c; Thilly et al. 1980a) is probably explained by the mechanism involved in the etiology of endemic cretinism, i.e., thyroid insufficiency occurring during the critical period of brain development covering the fetal life and the first years of life (Delange 1981). According to such a concept, cassava toxicity for the brain should be mediated by an elevated production of SCN, as described for the pathogenesis of goitre. However, the large amounts of HCN present in several cassava-based foodstuffs evoke the question of whether mental retardation in endemic goitre could result from a direct toxic action of HCN on the central nervous system.

In summary, two main questions arose from our previous observations on the *Role of cassava in the etiology of endemic goitre and cretinism* (Ermans et al. 1980a).

- What are the nutritional prerequisites for cassava to induce the development of endemic

Table 1. Presumed epidemiological and nutritional characteristics of the four regions investigated in Zaire when the studies were initiated.<sup>a</sup>

Regions	Goitre	Iodine deficiency	Level of cassava intake	Malnutrition
Ubangi (Gemena)	+++	+++	+++	—
Bas Zaire (Kisantu))	—(?)	?	+++	±(?)
Kivu (Kabare)	?	?	+++	+++
Kinshasa (control)	—	—(?)	—	—
Brussels (control)	—	—	—	—

<sup>a</sup>Symbols denote: +++, extremely frequent; —, absent; ?, unknown.

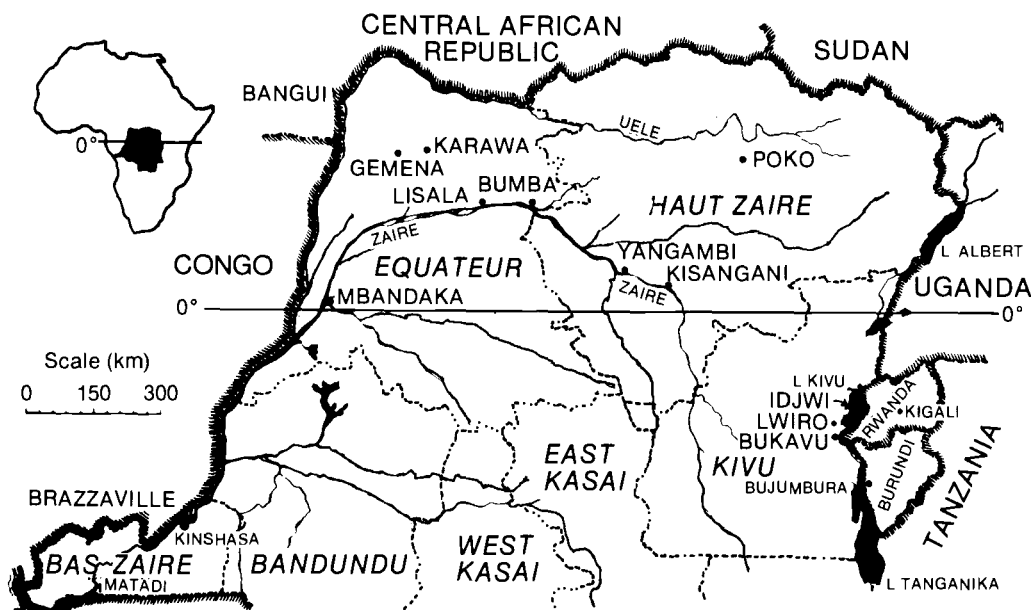


Fig. 1. Geographic location of the four regions investigated in Zaire.

goitre and cretinism in humans? More specifically, what are the respective roles of iodine and of protein calorie intakes in the presence of a cassava-based diet?

- What mechanisms are responsible for mental retardation observed in endemic goitre? Is it caused indirectly by congenital thyroid failure or directly by a toxic action of HCN on the central nervous system?

Answers to these questions have important practical implications for the health and development of millions of people in developing countries whose staple diet is cassava. Indeed,

the possibility arises that overuse of cassava for nutritional purposes and economic reasons could create new diseases, including mental retardation, in currently unaffected areas by introducing a disequilibrium among the different constituents of the diet.

Our first objective, therefore, was to evaluate the nutritional conditions required in humans for cassava to induce endemic goitre and cretinism. This objective was reached by means of field studies performed in Zaire in four different regions. These regions were characterized by the presence or absence of goitre and of malnutrition and by different intake levels

Table 2. Groups of patients investigated in the four regions in Zaire and expected information.

Groups	Optimal sample size (number of patients)	Expected information and methodology
General population	2000-3000	Determination of prevalences of goitre, cretinism, and malnutrition. Estimation of iodine, cassava, and protein intake levels. Epidemiological and biochemical methods. Nutritional surveys.
Young adults	100-200	Estimation of nutritional status in terms of iodine, cassava, and protein calorie intake. Biochemical methods.
Mothers at delivery and newborns	100	Evaluation of cassava toxicity for the thyroid. Biochemical methods.
Infants (0-3 years)	100	Evaluation of cassava toxicity for the thyroid. Biochemical methods.

of cassava and iodine. Table 1 summarizes the information available when these studies were initiated.

Fig. 1 shows the geographical location of these regions. Kinshasa was used as a control region and Ubangi has been described in detail previously (Ermans et al. 1980a). Kisantu is 120 km southwest of Kinshasa on the way to Matadi. The landscape is flat or slightly hilly, covered by savanna with isolated remains of forest. Bas Zaire was the first region to which cassava was introduced during the 16th or 17th century (Jones 1959). The people of this region are known to practice the most elaborate and efficient detoxification processes used in the country for removing linamarin from cassava. Malnutrition has recently appeared in Kisantu as a result of mass transfer of agricultural products to Kinshasa for commercial purposes. Another consequence of food shortage in the area is that the inhabitants have progressively simplified cassava processing and, in particular, have shortened the period of soaking (chapter 5).

Kabare in Kivu is close to the southwestern shore of Lake Kivu. This area has been extensively studied by Vis and coworkers (Vis 1963, 1968, 1969; Vis et al. 1969; P. Hennart in preparation). The area is characterized by extreme protein calorie malnutrition, the presence of which has been compounded by a population explosion. In 1969, Vis et al. reported that cassava was a minor constituent of the diet, representing, on the average, 17.6% of the annual caloric intake. In 1972, Thilly et al. reported

that the prevalence of goitre in this region was only 1.8% of the total population despite a state of iodine deficiency. Over the last 8 years, the research team at Kivu has observed a progressive increase in the consumption of cassava, as a consequence of food shortage, and a concomitant increase in goitre, although no formal epidemiological survey on the prevalence of goitre was conducted.

In the four regions investigated in Zaire, four different groups of patients have been studied. These groups are defined in Table 2 with the information expected from investigations conducted on each of them.

No study could be carried out in the general population of Kinshasa, however, and investigations in this region were limited to the group of mothers and newborns. They were carried out mainly in a clinic attended by the upper socioeconomic class of Kinshasa with no nutritional problems. A limited study on mothers and newborns was also conducted in another maternity clinic located in a poor suburb of Kinshasa attended by the lower socioeconomic group for whom cassava was supposed to constitute a more important part of the diet.

Our second objective, i.e., to evaluate the respective roles played by thyroid failure and by toxic effect of HCN on the central nervous system, was completed in experiments on rats. Groups of pregnant rats were fed various diets characterized by the presence or absence of iodine deficiency, and HCN and SCN overload. The study included the estimation of the

growth of the litter, the weight of the thyroid and of the brain, the serum levels of thyroxine ( $T_4$ ) and SCN and the brain content of total proteins, DNA, RNA, and lipids to assess the cellularity and the myelinization process.

Throughout the text, we have used the following abbreviations and symbols:

SCN — thiocyanate

HCN — cyanide

I — iodine or iodide

$T_3$  — triiodothyronine

$T_4$  — thyroxine

$rT_3$  — reverse triiodothyronine

PBI — protein bound iodine

TSH — thyrotropin (thyroid stimulating hormone)

TBPA — thyroxine binding prealbumin

TBG — thyroxine binding globulin.

The results reported in this monograph throw new light on the nutritional and environmental aspects of cassava toxicity.





## Introduction

A.M. ERMANS ET F. DELANGE

Des études antérieures effectuées au Zaïre ont montré que le manioc exerce une action antithyroïdienne chez l'homme et chez l'animal et qu'il joue un rôle déterminant dans l'étiologie du goitre et du crétinisme endémiques (Ermans et al. 1980a). Cette action résulte de la libération endogène de thiocyanate (SCN) à partir de la linamarine, un glucoside cyanogénique contenu dans le manioc, en particulier dans les tubercules (Bourdoux et al. 1980a).

Cependant, en dépit du fait que le manioc est consommé sur une large échelle dans les régions tropicales (Nestel 1973; Phillips 1974), il n'existe pas nécessairement de goitre et de crétinisme parmi toutes les populations pour lesquelles le manioc constitue la base de l'alimentation (Kelly et Snedden 1960; Kochupillai et al. 1980; Medeiros-Neto et Dunn 1980).

Une des explications possibles de l'absence d'action goitrigène du manioc dans certaines populations pourrait être que ces populations disposent d'un apport iodé élevé. En effet, dans les régions investiguées au Zaïre (île Idjwi, Kivu et région de l'Ubangi, Equateur), l'action antithyroïdienne du manioc a été observée parmi des populations soumises à une carence iodée sévère (Delange et al. 1968; Ermans et al. 1969, 1980a; Thilly et al. 1972;

Delange 1974). De plus, la correction de la carence iodée grâce à des injections d'huile iodée lentement résorbable a été suivie de la disparition du goitre et du crétinisme endémiques ainsi que d'une normalisation de la fonction thyroïdienne (Thilly et al. 1973; Thilly 1979; Lagasse et al. 1980b). Cette dernière observation est en accord avec des résultats expérimentaux montrant que chez le rat, l'effet goitrigène provoqué par l'administration de quantités importantes de SCN peut être entièrement supprimé grâce à une supplémentation en iode (Ermans et al. 1980b).

Des études effectuées en Sicile ont montré que le SCN pouvait avoir une action goitrigène même lorsque l'apport iodé n'est pas aussi drastiquement abaissé qu'au Zaïre (Delange et al. 1978).

Par conséquent, la question se pose de savoir à partir de quel niveau d'apport en iode l'ingestion de précurseurs de SCN tels que le manioc perturbe le métabolisme iodé intra-thyroïdien et joue un rôle déterminant dans l'étiologie du goitre endémique.

Un autre facteur nutritionnel qui pourrait être impliqué dans le caractère goitrigène du manioc chez l'homme est l'apport protéocalorique étant donné que la conversion endogène de cyanure (HCN) en SCN requiert la présence d'acides aminés soufrés (Ermans et al. 1972; Oke 1973; Osuntokun 1973; Barrett et al. 1978; Bourdoux et al. 1980a). Expérimentalement chez le porc, la carence protéique protège vis-à-vis de l'action antithyroïdienne du manioc grâce à la diminution de la conversion de HCN en SCN qu'elle entraîne (Tewe 1976). Il a également été démontré expérimentalement chez l'animal qu'en présence d'une insuffisance de l'apport protéocalorique, il existe une limitation dans le processus de goitrogenèse résultant de l'administration de régimes goitrigènes (Aschkenazy et al. 1962; Cowan et Margossian 1966; Shrader et al. 1977).

L'observation d'un retard de développement psychomoteur chez des jeunes enfants de l'endémie goitreuse de l'Ubangi qui ne présentent pas les autres stigmates du crétinisme endémique (Lagasse et al. 1980c; Thilly et al. 1980a) s'explique probablement par le même mécanisme que celui intervenant dans l'étiologie du crétinisme, à savoir une insuffisance thyroïdienne survenant durant la période critique pour le développement cérébral s'étendant de la vie foetale aux premières années de la vie (Delange 1981). Selon cette hy-

Tableau 1. Caractéristiques épidémiologiques et nutritionnelles supposées des quatre régions investiguées au Zaïre au moment où les études ont été entamées.<sup>a</sup>

Régions	Goitre	Carence iodée	Consommation de manioc	Malnutrition
Ubangi (Gemena)	+++	+++	+++	-
Bas-Zaïre (Kisantu))	-(?)	?	+++	±(?)
Kivu (Kabare)	?	?	+++	+++
Kinshasa (contrôle)	-	-(?)	-	-
Bruxelles (contrôle)	-	-	-	-

<sup>a</sup>Signification des symboles : + + +, extrêmement fréquent; -, absent; ?, inconnu.

pothèse, la toxicité du manioc pour le cerveau devrait être secondaire à la surcharge en SCN, comme il a été montré pour la pathogénie du goitre. Toutefois, compte tenu des quantités importantes de HCN contenues dans certains aliments dérivés du manioc, la question peut se poser de savoir si le retard mental observé dans le goitre endémique pourrait résulter d'une action toxique directe du HCN sur le système nerveux central.

En résumé, deux questions principales se posent vu les observations que nous avons effectuées concernant le rôle joué par le manioc dans l'étiologie du goitre et du crétinisme endémiques (Ermans et al. 1980a) :

- Quelles sont les conditions nutritionnelles requises chez l'homme pour que la consommation de manioc provoque l'apparition de goitre et de crétinisme endémiques ? Plus précisément, quels sont les rôles respectifs des apports iodé et protéo-calorique en présence d'une alimentation à base de manioc ?

- Quels sont les mécanismes responsables du retard mental dans le goitre endémique ? Ce retard est-il la conséquence d'une hypothyroïdie congénitale ou d'une action toxique directe du HCN sur le système nerveux central ?

Les réponses apportées à ces questions ont des implications importantes en ce qui concerne la santé et le développement de larges fractions de populations du Tiers-Monde qui survivent essentiellement grâce à une alimentation à base de manioc. Il est en effet concevable qu'une augmentation drastique de la consommation de manioc faisant suite à la situation socio-économique précaire des populations concernées provoque l'apparition de nouvelles conditions pathologiques, y compris le retard mental, dans des régions épargnées jusqu'à présent.

Le premier objectif du présent travail a donc été d'évaluer les conditions nutritionnelles requises chez l'homme pour que le manioc pro-

voque le développement de goitre et de crétinisme endémiques. Cet objectif a été atteint grâce à des études effectuées sur le terrain au Zaïre dans quatre régions différentes. Ces régions sont caractérisées par la présence ou l'absence de goitre et de malnutrition et par des différences dans les apports nutritionnels en manioc et en iode. Le Tableau 1 résume les informations qui étaient disponibles dans ces quatre régions au moment où nos travaux ont été entamés.

La Fig. 1 (voir la version anglaise) montre la localisation géographique des quatre régions. Kinshasa a été utilisée comme région contrôle au Zaïre. La région de l'Ubangi a été décrite en détails antérieurement (Ermans et al. 1980a).

Kisantu est situé à 120 km au sud-ouest de Kinshasa sur la route de Matadi. Le paysage est plat ou caractérisé par de petites collines recouvertes de savane ou de résidus de forêt. Le Bas-Zaïre où le manioc a été introduit au cours des XVI<sup>e</sup> et XVII<sup>e</sup> siècles (Jones 1959), a la réputation d'être une région où les processus de détoxification du manioc, incluant le rouissage, sont les plus élaborés et efficaces. De la malnutrition est récemment apparue dans la région de Kisantu, faisant suite notamment au transfert massif de denrées alimentaires vers Kinshasa à des fins commerciales. Une autre conséquence des restrictions alimentaires dans cette région est que les processus de détoxification du manioc utilisés par les habitants ont été progressivement simplifiés. En particulier, la durée du rouissage a été diminuée (voir aussi chapitre 5).

Kabare est situé au Kivu à proximité de la rive sud-ouest du lac Kivu. Cette région a fait l'objet d'études approfondies par Vis et al. (Vis 1963, 1968, 1969; Vis et al. 1969; P. Hennart en préparation). La région est caractérisée par une malnutrition protéo-calorique extrêmement sévère qui va en s'aggravant au cours des années, notamment en raison de l'explosion démographique. En 1969, Vis et al. ont décrit que

Tableau 2. Groupes de patients investigués dans les quatre régions étudiées au Zaïre et informations attendues.

Groupes	Taille optimale de l'échantillon (nombre de patients)	Informations attendues et méthodes utilisées
Population générale	2000-3000	Détermination des prévalences de goitre, de crétinisme et de malnutrition. Estimation des apports iodé, protéo-calorique et de manioc. Méthodes épidémiologiques et biochimiques. Enquêtes nutritionnelles.
Jeunes adultes	100-200	Estimation du statut nutritionnel en termes d'apports iodé et protéique et de manioc. Méthodes biochimiques.
Mères à l'accouchement et nouveau-nés	100	Appréciation de la toxicité du manioc pour la fonction thyroïdienne. Méthodes biochimiques.
Enfants âgés de 0 à 3 ans	100	Appréciation de la toxicité du manioc pour la fonction thyroïdienne. Méthodes biochimiques.

le manioc ne constituait qu'un des composants mineurs de l'alimentation. Il représentait 17,6 % de l'apport calorique annuel moyen. En 1972, Thilly et al. ont montré que la prévalence du goitre à Kabare n'était que de 1,8 % de la population totale en dépit de l'existence d'une carence iodée manifeste. Au cours des huit dernières années, l'équipe du Kivu a observé une augmentation progressive de la consommation de manioc en raison des problèmes d'alimentation. L'équipe a également noté que le goitre devenait une observation clinique fréquente dans la population. Toutefois, il n'a pas été effectué d'enquête épidémiologique systématique concernant la prévalence du goitre.

Dans chacune des quatre régions investiguées au Zaïre, quatre groupes différents de patients ont été étudiés. Les groupes sont définis dans le Tableau 2, ainsi que les informations attendues sur la base des investigations effectuées dans chacun d'eux.

Toutefois, il n'a pas pu être effectué d'études parmi la population générale à Kinshasa et les investigations conduites dans cette région ont été limitées aux mères à l'accouchement et aux nouveau-nés. Ces enquêtes ont été effectuées principalement dans une clinique fréquentée par une classe socio-économique élevée de Kinshasa, qui ne présente pas de problèmes nutritionnels. Une étude limitée a également été pratiquée dans une autre maternité située dans un faubourg de Kinshasa à niveau socio-économique plus bas et pour lequel le manioc semblait constituer une part plus importante de l'alimentation.

Le deuxième objectif du présent travail, à savoir l'évaluation des rôles respectifs joués par l'insuffisance thyroïdienne et par l'effet toxique direct du HCN sur le système nerveux central a été atteint grâce à des études expérimentales effectuées chez l'animal. Des groupes de rates gravides ont été soumises à différents régimes alimentaires caractérisés par l'absence ou la présence de carence iodée et de surcharge en HCN et en SCN. L'étude a consisté en une estimation de la croissance pondérale des rats, du poids de leurs thyroïdes et cerveaux, des concentrations sériques de thyroxine ( $T_4$ ) et de SCN et du contenu des cerveaux en protéines, DNA, RNA et lipides dans le but d'apprécier la cellularité et le processus de myélinisation.

Dans le texte, nous avons utilisé les abréviations suivantes :

SCN — thiocyanate  
HCN — cyanure  
I — iode ou iodure  
 $T_3$  — triiodothyronine  
 $T_4$  — thyroxine  
 $rT_3$  — triiodothyronine inverse  
PBI — iode protéique plasmatique  
TSH — thyreostimuline  
TBPA — thyroxine binding prealbumin  
TBG — thyroxine binding globulin.

Les résultats des études décrites dans la présente monographie apportent une série d'informations nouvelles concernant les relations existantes entre la toxicité du manioc et les conditions d'environnement nutritionnel.



## Chapter 1

# Biochemical and Statistical Methods

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STEIRTEGHEM

Most of the chapters of this monograph report results from epidemiological studies and biochemical investigations of thyroid function, SCN metabolism, and dietary supplies of iodine, HCN, and proteins. The comparison of and the relationships between the results in the different groups of patients have been assessed by several statistical methods.

This chapter provides a detailed description of the biochemical and statistical methods used.

## Biochemical Methods

### Assessment of thyroid function

*Preparation of  $T_3$ - and  $T_4$ -free serum:* To 100 ml normal human serum were added 20 g Norit 0L (Hopkin and Williams, Essex, England). The mixture was kept at 4°C under magnetic stirring for 24 hours, centrifuged twice at  $25\,000 \times g$  to remove the Norit, and sequen-

tially filtered through Millex-HA 0.45  $\mu\text{m}$  and Millex-HA 0.22  $\mu\text{m}$ . The removal of iodothyronines was monitored with  $T_3$  or  $T_4$  of high specific activity.

*Serum human  $T_4$ :* To 10  $\mu\text{l}$  of standard (0, 1.25, 2.5, 5, 10, and 20  $\mu\text{g}/\text{dl}$  in human  $T_4$ -free serum) or sample were added 100  $\mu\text{l}$   $^{125}\text{I}$ - $T_4$  (specific activity higher than 1200  $\mu\text{Ci}/\mu\text{g}$ , Amersham International Ltd, Amersham, England) in barbital buffer (pH 8.6; 0.05 M; 8-anilino-naphthalene-sulfonic acid 0.03%; thimerosal 0.01%; bovine gamma globulins 0.75%), and 300  $\mu\text{l}$  antiserum to  $T_4$  (final dilution 1 : 22 000) in barbital buffer. The mixture was incubated for 2.5 hours at room temperature (ca. 20°C). Bound and free ligands were separated by addition of 2 ml 18% polyethylene glycol 6000 (PEG 6000) in barbital buffer (pH 8.5; 0.09 M; bovine serum albumin 0.1%). Thereafter, the tubes were centrifuged for 20 min at room temperature and the supernate was discarded. The final dilution of 1 : 22 000 gave, after blank subtraction, a zero binding of about 55% of total radioactivity. Quality control was done with three different pools of sera (low, normal, and high). For a serum sample of 5.6  $\mu\text{g}/\text{dl}$ , coefficients of variation (CV) were 3.5% for intraassay and 5.6% for interassay.

*Serum human  $T_3$ :* To 50  $\mu\text{l}$  of standard (0, 25, 50, 100, 200, and 400 ng/dl in human  $T_3$ -free serum) or sample were added 200  $\mu\text{l}$   $^{125}\text{I}$ - $T_3$  (specific activity higher than 1200  $\mu\text{Ci}/\mu\text{g}$ , Amersham International Ltd) in barbital buffer (pH 8.6; 0.05 M; thimerosal 0.01%; bovine gamma globulins 0.75%), and 200  $\mu\text{l}$  antiserum to  $T_3$  (final dilution 1 : 50 000). The mixture was incubated for 2 hours at 37°C. Bound and free ligands were separated by addition of 2 ml 18% PEG 6000 in barbital buffer (as for  $T_4$ ). Thereafter, the tubes were centrifuged for 20 min at room temperature and the supernate was discarded. The final dilution of 1 : 50 000 gave, after blank subtraction, a zero binding of about 40% of total radioactivity. Quality control was done with three different pools of sera (low, normal, and high). For a serum sample of 140 ng/dl, CVs were 4.0% intraassay and 7.0% interassay.

*Serum human TSH:* The reagents were provided by the National Institute for Arthritis, Metabolism and Digestive Diseases (NIAMDD), National Institute of Health (NIH), Bethesda, MD, USA. We gratefully acknowledge this

contribution. To 100  $\mu\text{l}$  of standard (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50  $\mu\text{U/ml}$ ) or sample were added 100  $\mu\text{l}$  phosphate buffer (0.02 M; pH 7.4; sodium azide 0.2%, EDTA 0.07%; NaCl 0.9%; bovine serum albumin 0.2%; HCG 0.25 IU), 100  $\mu\text{l}$   $^{125}\text{I}$ -TSH (specific activity higher than 120  $\mu\text{Ci}/\mu\text{g}$ ), obtained by the method of Redshaw and Lynch (1974), in phosphate buffer, and 100  $\mu\text{l}$  antihuman-TSH (AFP) at a final dilution of 1 : 2 000 000. The mixture was incubated for 4 days at 4°C. Thereafter, 100  $\mu\text{l}$  0.4% normal rabbit serum and 100  $\mu\text{l}$  2% antirabbit gamma globulins were added and the mixture was further incubated at 4°C for 24 hours. All tubes were centrifuged at 4°C for 30 min, and the supernate was discarded. This procedure, after blank subtraction, gave a zero binding of about 50% of total radioactivity. Three control samples (5, 15, and 30  $\mu\text{U/ml}$ ) were run with reference material 68/38 provided by WHO International Laboratory for Biological Standards, Holly Hill, Hampstead, London, for which we are grateful. For a serum sample of 11.5  $\mu\text{U/ml}$ , CVs were 6.0% intraassay and 9.1% interassay.

*Spot human  $T_4$* : For the preparation of the standards, heparinized blood obtained from a normal person was centrifuged and the supernate discarded. The erythrocytes were washed three times with an equal volume of NaCl (0.9%) and the supernate was discarded. Equal volumes of erythrocytes and  $T_4$  standards (0, 1.25, 2.5, 5, 10, and 20  $\mu\text{g/dl}$  in human  $T_4$ -free serum) were mixed and spotted on filter paper (Schleicher and Schull 2992 or 903) in 25- $\mu\text{l}$  aliquots.

One 6-mm spot of standard or sample, 100  $\mu\text{l}$   $^{125}\text{I}$ - $T_4$  (as for serum  $T_4$ ) in barbital buffer (as for serum  $T_4$ ), and 300  $\mu\text{l}$  antiserum to  $T_4$  at a final dilution of 1 : 88 000 in barbital buffer were incubated overnight at 4°C. Bound and free ligands were separated by addition of 2 ml 18% PEG 6000. The tubes were then centrifuged for 20 min at room temperature and the supernate was discarded. This procedure, after blank subtraction, gave a zero binding of about 40% of total radioactivity. Quality control was done with three different pools of sera (low, normal, and high). For a sample of 7.4  $\mu\text{g/dl}$ , CVs were 5.5% intraassay and 8.9% interassay.

The coefficient of correlation between spot  $T_4$  and serum  $T_4$  in 197 samples was  $r = 0.929$  ( $P < 0.001$ ).

*Spot human TSH*: For the preparation of standards, erythrocytes were obtained as for spot  $T_4$ , mixed with an equal volume of TSH standards (0, 12.5, 25, 50, and 100  $\mu\text{U/ml}$  in human serum from hyperthyroid patients), and spotted on filter paper as for spot  $T_4$ .

One 6-mm spot of standard or sample, 100  $\mu\text{l}$  phosphate buffer (0.02 M, pH 7.4; sodium azide 0.2%; EDTA 0.07%; NaCl 0.9%; HCG 0.30 IU; human TSH-free serum 20%), and 100  $\mu\text{l}$  antihuman-TSH (AFP) at a final dilution of 1 : 1 000 000 in phosphate buffer were incubated overnight at room temperature. Thereafter, 100  $\mu\text{l}$   $^{251}\text{I}$ -TSH (as for serum TSH) were added and the mixture was incubated at room temperature for 24 hours. Bound and free ligands were separated by the addition of 2 ml 18% PEG 6000 (as for serum  $T_4$ ). The tubes were then centrifuged for 20 min at room temperature and the supernate was discarded. This procedure, after blank subtraction, gave a zero binding of about 35% of total radioactivity. Quality control was done with two different samples (20 and 50  $\mu\text{U/ml}$ ) with reference material 68/38. For a spot sample of 20.0  $\mu\text{U/ml}$ , CVs were 8.3% intraassay and 12.4% interassay.

The coefficient of correlation between spot TSH and serum TSH in 460 samples was  $r = 0.962$  ( $P < 0.001$ ).

*Serum rat  $T_4$* : To 10  $\mu\text{l}$  of standard (0, 0.62, 1.25, 2.5, 5, and 10  $\mu\text{g/dl}$  in rat T-free serum) or sample was added 100  $\mu\text{l}$   $^{125}\text{I}$ - $T_4$  in barbital buffer (as for serum human  $T_4$ ), and 300  $\mu\text{l}$  antiserum to  $T_4$  (final dilution 1 : 44 000) in barbital buffer. The mixture was incubated overnight at 4°C. Bound and free ligands were separated by the addition of 100  $\mu\text{l}$  0.4% normal rabbit serum and 100  $\mu\text{l}$  2% antirabbit gamma globulins. Subsequently, the mixture was incubated at 4°C for 24 hours. Thereafter, the tubes were centrifuged and the supernate was discarded. This procedure, after blank subtraction, gave a zero binding of about 45% of total radioactivity. Quality control was the same as for human serum  $T_4$ .

*Thyroidal uptake of radioiodine*: Uptake of radioiodine was determined according to the method described by Bourdoux et al. (1978). The rates of thyroidal uptake of radioiodine ( $^{131}\text{I}$ ) were determined with a Pitman portable probe (model 235). The detector was a scintillation system consisting of a sodium iodide (NaI) crystal ( $2.54 \times 2.54$  cm) coupled to a 2.54-cm photomultiplier tube. The lead collimator

(diameter, 6.25 cm; length 6.25 cm) was set to a distance of 1.27 cm from the crystal. Distance between the crystal and the subject's neck was 25 cm.

### Assessment of HCN, SCN, and iodine dietary supplies

**HCN:** Peeled cassava root, as well as other food samples, were rapidly homogenized without added liquid. An aliquot of the homogenate was weighed and transferred into a 25-ml Erlenmeyer flask with a centre well containing 0.5 ml 1 M NaOH, and 5 ml acetate buffer (0.1 M; pH 5.5) were then added. The flask was closed hermetically and incubated at 37°C for 20 hours with agitation. The hydrocyanic acid liberated by autolysis was trapped in the centre well and assayed in an aliquot according to the modified procedure of Lambert et al. (1975): 0.1 ml of standard (5, 3.33, 1.67, 0.83, and 0.42  $10^{-4}$  M cyanide/l) or sample was mixed with 0.9 ml 0.1 M NaOH, 0.5 ml 1.0 M acetic acid, 5 ml succinimide/N-chlorosuccinimide reagent (succinimide 0.25% and N-chlorosuccinimide 0.025%), and 1 ml barbituric acid/pyridine reagent (barbituric acid 6% and pyridine–water, 30 : 70 v/v). After 10 min, optical density was read against blank at 580 nm.

**SCN:** SCN was measured according to the procedure of Aldridge (1945) as modified by Michajlovskij and Langer (1958): 0.5 ml serum, urine, or milk (the latter previously defatted with chloroform) were mixed with 2 ml 15% trichloroacetic acid and centrifuged for 10 min. An aliquot of the supernate (1.5 ml) was transferred to a glass spectrophotometer tube; standards were prepared by mixing 0.3 ml of KSCN solutions (0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg SCN/dl) with 1.2 ml 15% trichloroacetic acid. The following reagents were then added sequentially to each tube: 0.2 ml saturated bromine water, 0.2 ml 4%  $\text{As}_2\text{O}_3$  in 2% NaOH, 1.6 ml 1% benzidine  $\cdot 2\text{HCl}$  in water, and 2 ml pyridine. After 30 min, optical density was read against blank at 525 nm.

**Iodide:** Iodide in urine was determined by the method of Riley and Gochman (1964) with a Technicon Autoanalyzer II.

**Calculation and expression of the results:** All samples measured by radioimmunoassay were assayed in duplicate or triplicate and results were calculated in a logit-log system (Rodbard et al.

1968) or spline function system (Marschner et al. 1974). The results reported for HCN analysis are the mean of four to six replicate assays.

### Assessment of the protein dietary supply

Serum total protein and albumin concentrations were measured on a Centrifichem Analyzer (Union Carbide Corporation, NY, USA).

Total protein was measured using the biuret reaction (Gornall et al. 1949). The biuret stock reagent was prepared by dissolving 15.0 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 70–80 ml of water and 45.0 g  $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  in 600–700 ml of water. The copper sulphate solution was added to the tartrate solution with stirring and the resulting mixture was made up to 1000 ml with water. The working reagent was obtained by diluting the stock solution fivefold with a KI solution (5 g KI/l of 0.25 M NaOH) and was stored in dark polyethylene bottles. The blank reagent was prepared by dissolving 9.0 g  $\text{NaKC}_4\text{H}_4\text{O}_6$  in 500 ml of water; after addition of 8.0 g NaOH and 5.0 g KI, the solution was made up to 1000 ml with water.

Albumin was measured by the bromocresolgreen dye method (Rodkey 1965). The reagent was commercially available from Union Carbide Corporation and contained: 52.5 mg bromocresolgreen, 0.95 ml 23-lauryl ether (25%), 2.99 g citric acid monohydrate, and 675 mg sodium hydroxide. The albumin blank reagent was an aqueous solution of 8.1 g/l citric acid and 17.1 g/l trisodium dihydrate.

The assays were standardized with commercially available reagents (Calibrate, General Diagnostics, NJ, USA). Internal quality control was monitored with commercial reagents (Precinorm U and Precimat, Boehringer Mannheim Corporation, Mannheim, Germany).

Table 3 summarizes the conditions used for the total protein and albumin assays on the centrifugal fast analyzer (Koch et al. 1974; Savory et al. 1976).

Creatinine concentration was measured on a Technicon Autoanalyzer by the method of Jaffe (1886) with commercially available reagents (Chasson et al. 1961).

### Statistical Methods

#### Prevalence of goitre

The criteria used for defining and grading goitre in our studies were those proposed by

Table 3. Conditions used for the total protein and albumin assays on the centrifugal fast analyzer.

	Total protein	Albumin
Sample volume ( $\mu$ l)	10	5
Flush volume (NaCl 90 g/l)( $\mu$ l)	55	50
Reagent volume ( $\mu$ l)	350	350
Program	kinetic assay	end point
Temperature ( $^{\circ}$ C)	30	30
Wavelength (nm)	550	620
Time delay (sec)	3	3
Time interval (min)	1	1
Blank correction	- <sup>a</sup>	- <sup>a</sup>

<sup>a</sup>Stored values for blank absorbances are automatically subtracted with the "hold blank" program.

the Pan American Health Organization (Querido et al. 1974), slightly modified by our team (Delange et al. 1969; Lagasse et al. 1980a; Thilly et al. 1980b). We standardized the prevalence rates of goitre observed in the three rural populations in Zaire for age and sex by calculating the specific goitre rates in each age group and sex, and computing the total rate that would occur if these specific rates were applied to a "standard population." The standard population used consisted of the 328 089 subjects examined in Ubangi from October 1974 to December 1977 (Lagasse et al. 1980b).

## Growth curves

The growth curves for the populations of the three rural areas studied in Zaire were established by plotting the median values obtained for weight and height as a function of age and sex. The curves established for normal Belgian subjects by Wachholder and Graffar (1973) were used as controls.

## Means and comparison of means

Most of the results obtained for the variables measured in the different groups of patients investigated are reported as the arithmetic means ( $\bar{x}$ )  $\pm$  the standard error of the means (SEM), where SEM is given by:

$$SEM = S/\sqrt{n} = \left[ \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{(n-1)}} \right] / \sqrt{n} \quad (1)$$

where S is the standard deviation (SD).

The reason for showing SEM in the tables and figures of this monograph, instead of SD,

is that SEM is the parameter of dispersion that intervenes directly in the tests of probability used for the comparison of means expressed in their more simple form, for example in equation 2:

$$t_d' = (\bar{x}_1 - \bar{x}_2) / \sqrt{(SEM_1^2 + SEM_2^2)} \quad (2)$$

The means obtained in two groups of samples were compared using the Student's t or Cochran t' tests, after comparison of the variances ( $s^2 = SD^2$ ) of the two samples.

The statistics t and t' were used to calculate the probability associated with the "null hypothesis" ( $H_0$ ) that the means of the two populations ( $\mu_1$  and  $\mu_2$ ) from which samples have been collected are identical ( $H_0: \mu_1 = \mu_2$ ).

Two situations were possible: either the samples had common variances or they did not. This could be assessed by comparing the variances of the samples and calculating Fisher's F where:

$$F = (\text{larger } s^2 / \text{smaller } s^2) \quad (3)$$

Fisher (1924) produced the F distribution when the two variances are those of two independent samples extracted at random from normally distributed populations with a common variance,  $\sigma^2$ . If the probability for F was greater than a predetermined significance level of 0.05, we accepted the hypothesis of a common variance; if the probability was less than or equal to 0.05, we rejected the hypothesis.

Where the hypothesis of common variance was accepted, we calculated Student's t, based on the pooled variance estimate for (2/d) with ( $n_1 + n_2 - 2$ ) degrees of freedom:

$$t_d = (\bar{x}_1 - \bar{x}_2) / \sqrt{(s^2/n_1) + (s^2/n_2)} \quad (4)$$

where  $s^2$  is the weighted average of the sample variances and is the best estimator for the unknown, common, variance of the population ( $\sigma^2$ ).

Where the common variance hypothesis had to be rejected, i.e., the variances were different, t could not be computed as in equation 4 and an approximation was computed from:

$$t_d' = (\bar{x}_1 - \bar{x}_2) / \sqrt{(s_1^2/n_1) + (s_2^2/n_2)} \quad (5)$$

This equation is equivalent to equation 2 (Snedecor and Cochran 1967). This statistic t' is not distributed as Student's. However, the

probability for  $t'$  can be approximated by treating it as  $t$ , but with different degrees of freedom (Cochran 1964):

$$df = \frac{[(s_1^2/n_1) + (s_2^2/n_2)]^2}{[(s_1^2/n_1)/(n_1 - 1)] + [(s_2^2/n_2)/(n_2 - 1)]} \quad (6)$$

As we were testing the null hypothesis that the means of the two populations were equal ( $H_0: \mu_1 = \mu_2$ ), we only used a two-tailed probability associated with each value of  $t$  or  $t'$ .

In this monograph, three levels of significance for differences between two means have been used:  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , shown by \*, \*\*, and \*\*\*.

### Coefficients of correlation (r)

The Pearson product-moment correlation coefficient ( $r$ ) has been used in this monograph to measure the strength of the relationships between two variables, i.e., the goodness of fit

of a linear regression line to the data, and, when  $r$  is squared, the proportion of variance in one variable explained by the other (Yule 1907). Two-tailed significance tests have been calculated for each coefficient and were derived (Snedecor and Cochran 1967) from:

$$t = r[\sqrt{(n - 2)/(1 - r^2)}] \quad (7)$$

using Student's  $t$  with  $(n - 2)$  degrees of freedom.

### Computer analysis

Most of the individual results collected throughout our studies have been stored in and treated by the CDC 6600 computer of the Brussels Free University's Computing Center. An important part of the analysis has been performed using the statistical package for social sciences of this centre (Nie et al. 1975). We thank the Computing Center for their assistance.





## Chapter 2

# Epidemiology of Goitre and Malnutrition and Dietary Supplies of Iodine, Thiocyanate, and Proteins in Bas Zaire, Kivu, and Ubangi

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**Epidémiologie du goitre et de la malnutrition et apports alimentaires en iode, thiocyanate et protéines au Bas-Zaïre, au Kivu et en Ubangi — Résumé —** Dans le but de définir les conditions nutritionnelles requises chez l'homme pour que le manioc provoque l'apparition de goitre et de crétinisme endémiques, des études comparatives épidémiologiques, cliniques, nutritionnelles et biochimiques ont été effectuées au Bas-Zaïre, au Kivu et en Ubangi. Bruxelles a été utilisée comme région témoin.

Les prévalences de goitre sont de 3,0 % à Bruxelles, de 1,5 % au Bas-Zaïre, de 12,5 % au Kivu et de 76,8 % en Ubangi (Tableau 4). Le chiffre obtenu pour le Kivu est environ 10 fois plus

élevé que celui obtenu il y a environ 10 ans dans la même région. Cette hausse correspond à une augmentation importante de la consommation de manioc. L'étude des signes cliniques de malnutrition (Tableau 4), des courbes de poids et de taille (Fig. 3) et des concentrations sériques d'albumine (Fig. 4) en fonction de l'âge montre qu'il existe une malnutrition modérée au Bas-Zaïre, extrêmement sévère au Kivu et seulement occasionnelle en Ubangi.

L'apport alimentaire en iode apprécié par la concentration urinaire d'iodure est de 5,3  $\mu\text{g}/\text{dl}$  à Bruxelles (51,2  $\mu\text{g}/\text{jour}$ ), soit une valeur relativement basse par rapport aux normes internationales (100  $\mu\text{g}/\text{jour}$ ). Par rapport aux résultats obtenus à Bruxelles, l'apport iodé est à peine abaissé au Bas-Zaïre (4,0  $\mu\text{g}/\text{dl}$ ) mais fortement abaissé à la fois au Kivu (2,7  $\mu\text{g}/\text{dl}$ ) et en Ubangi (2,3  $\mu\text{g}/\text{dl}$ ). L'apport alimentaire en SCN apprécié par la concentration urinaire de SCN est de 0,60  $\text{mg}/\text{dl}$  à Bruxelles. Il est nettement supérieur dans les trois régions investiguées au Zaïre : 1,43  $\text{mg}/\text{dl}$  au Bas-Zaïre, 1,10  $\text{mg}/\text{dl}$  au Kivu et 1,82  $\text{mg}/\text{dl}$  en Ubangi. Ces taux élevés résultent d'une importante consommation de manioc dans ces trois régions où de 91 à 96 % des personnes interrogées ont consommé du manioc dans les 24 heures précédant l'interrogatoire. La forme sous laquelle le manioc est consommé est cependant très différente dans les trois régions : il s'agit essentiellement de manioc roui au Bas-Zaïre et de manioc non roui au Kivu et en Ubangi.

En conséquence des modifications respectives observées pour les concentrations urinaires d'iode et de SCN, le rapport urinaire I/SCN utilisé comme indice de la balance alimentaire en iode et en SCN diminue progressivement de 8,8 à Bruxelles, à 3,8 au Bas-Zaïre, 2,6 au Kivu et 1,9 en Ubangi.

En conclusion, ce travail montre qu'en présence d'une alimentation à base de manioc et d'un apport iodé faible, l'apparition de goitre endémique est liée de manière critique à la balance existant entre les apports en iode et en SCN. Il n'existe pas de goitre endémique aussi longtemps que le rapport urinaire I/SCN est supérieur au seuil critique de 3 à 4, mais le goitre devient hyperendémique compliqué par du crétinisme lorsqu'il est inférieur à 2 (Fig. 6). Ce travail montre également que la présence de malnutrition dans une population n'affecte pas le processus de conversion endogène de HCN en SCN au niveau des individus. Il montre enfin que l'introduction massive de manioc en présence d'une carence iodée peut provoquer l'apparition de goitre dans des populations qui en étaient antérieurement indemnes.

As reported in the Introduction, one of our main objectives has been to define the nutritional conditions required in humans for cassava to induce endemic goitre and cretinism. Special attention has been devoted to the roles of iodine and of protein calorie intake levels in the presence of a cassava-based diet.

The methodology used to reach this objective comprised comparative epidemiological, clinical, nutritional, and biochemical studies in Brussels (as a control) and in four regions in Zaire characterized by the presence or absence of endemic goitre and of malnutrition and by various levels of iodine, cassava, and protein calorie intake. The studies in Zaire were carried out in Kinshasa (as control), in Bas Zaire at Kisantu, Kivu at Kabare, and Ubangi at Gemena. The studies in Kinshasa were limited to mothers and newborns at delivery.

The purposes of the work described in this chapter were:

- To determine, on a clinical basis, the prevalences of goitre, cretinism, and malnutrition in the general population of Bas Zaire, Kivu, and Ubangi.
- To estimate the dietary supplies of iodine, SCN, and proteins in the same populations.
- To study the food habits of these populations and, in particular, the frequency and type of cassava-based foods.

## Methods

### Epidemiological surveys

The prevalences of goitre and cretinism were determined using the criteria recommended by the Pan American Health Organization (Querido et al. 1974), modified by Thilly et al. (1980b) and Lagasse et al. (1980a), and that of clinically detectable protein malnutrition using the criteria proposed by Vis (1963, 1969) and by P. Hennart (in preparation). Moreover, the patients' nutritional status was further assessed by comparing their weight-for-height with standards established by Stuart and Stevenson (1959) for normal North American subjects. According to these authors, a weight-for-height lower than 80% of the median (or 50th percentile) of the standard curve indicates malnutrition.

The surveys were carried out in randomly selected samples of the total populations of the three regions, which consisted of 2599 inhabitants of Bas Zaire, 2687 inhabitants of Kivu, and 4707 inhabitants of Ubangi. The study in Ubangi was limited to the Ngwaka (or Ngbaka)

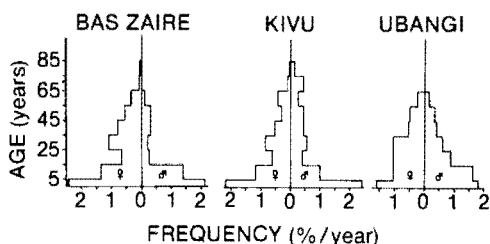


Fig. 2. Distribution by age and sex in the populations studied in Bas Zaire, Kivu, and Ubangi.

ethnic group for whom unsoaked cassava is the main staple food (Simons-Gérard et al. 1980). Particular attention was paid to the prevalence of malnutrition in children aged 2–9 years, as this age group is the most sensitive to malnutrition (Vis 1963).

Finally, the prevalence of splenomegaly, used as an index of chronic infection (mainly malaria), was estimated in children aged 5–14 years.

Fig. 2 shows the frequency distribution by age and sex of the populations surveyed. In all three areas, the number of females was higher than the number of males, the fractions of the populations aged less than 15 years were 40.5% in Bas Zaire, 44.2% in Kivu, and 43.0% in Ubangi. Thus, the samples studied were representative of the total populations investigated and were comparable from one region to another.

The prevalence of goitre was not determined in a sample of the total population in Brussels; however, a survey of 1417 Belgian schoolchildren aged 6–16 indicated a prevalence of 2% in the western part of the country, of 10% in the eastern part, and of 3% in Brussels (C.H. Thilly et al. unpublished). Previous data collected by Brull and Dewart (1956) in 54 237 males (aged 18 years) entering the army showed a mean prevalence of goitre of 1.3%. Based on these data, we assumed that the prevalence of goitre in Brussels was not higher than 3%.

### Biochemical assessment of the dietary supplies of iodine, SCN, and proteins

The dietary supplies of iodine and SCN in the populations were estimated from the daily urinary excretions and urinary concentrations of iodine and SCN and by the urinary I/creatinine and SCN/creatinine ratios, as proposed by Querido et al. (1974). Collections of

24-hour urine were performed in young adults. The balance between the iodine and SCN supplies has been estimated, as reported previously (Ermans et al. 1980a), by the urinary I/SCN ratio (iodine expressed in  $\mu\text{g}/\text{dl}$  and SCN expressed in  $\text{mg}/\text{dl}$ ).

The dietary supply of proteins in the populations was estimated by the changes in serum concentration of albumin as a function of age and sex and by the daily urinary excretion of creatinine.

As reported in chapter 1, serum albumin was determined by the bromocresolgreen (BCG) method, except for samples collected by P. Hennart and H.L. Vis in Kivu during a previous study (unpublished). In the latter, albumin was determined by paper electrophoresis (Wolfson et al. 1948; Sonnet and Rodhain 1952) and was systematically lower than by the BCG method. However, the results obtained by the two methods in 118 samples were significantly correlated ( $r = 0.695$ ,  $P < 0.001$ ).

## Nutritional surveys

As Simons-Gérard et al. (1980) reported, the conditions in the field did not allow us to perform quantitative nutritional surveys. We therefore conducted qualitative surveys using questionnaires. Each person surveyed was requested to list the food items eaten during the previous 24 hours.

Of 1703 unselected subjects interviewed, 977 were in Ubangi and 701 in Kivu; because the purpose of the survey was misunderstood by the population in Bas Zaire and most of the inhabitants refused to reply to the questions, the

survey was limited to 25 women aged 20–60 years. However, given that the women prepare meals for the whole family and that their replies were practically identical, we felt that this information might be representative of the population in Bas Zaire.

The large majority of persons questioned in Ubangi, Kivu, and Bas Zaire were Ngwaka, Bashi, and Bakongo respectively. All were living on a self-subsistent economy based on agriculture.

## Results

### Epidemiological surveys

The prevalences of goitre, cretinism, malnutrition, and splenomegaly in the three regions investigated in Zaire are compared in Table 4. The prevalence of goitre was 1.5% in Bas Zaire, 12.5% in Kivu, and 76.8% in Ubangi where about 50% of the total population had visible goitre and 20% had nodular goitre. Endemic cretinism was not found in Bas Zaire or in Kivu; its prevalence was 4.7% in Ubangi where 91% of the endemic cretins were myxedematous.

Clinically detectable malnutrition was extremely frequent in Kivu. In particular, 26.0% of the children aged 2–9 years had a weight-for-height lower than 80% of the 50th percentile for a normal North American population, 7.2% had depigmentation of the skin, and 9.0% had edema. The prevalence of these symptoms was markedly lower in Bas Zaire

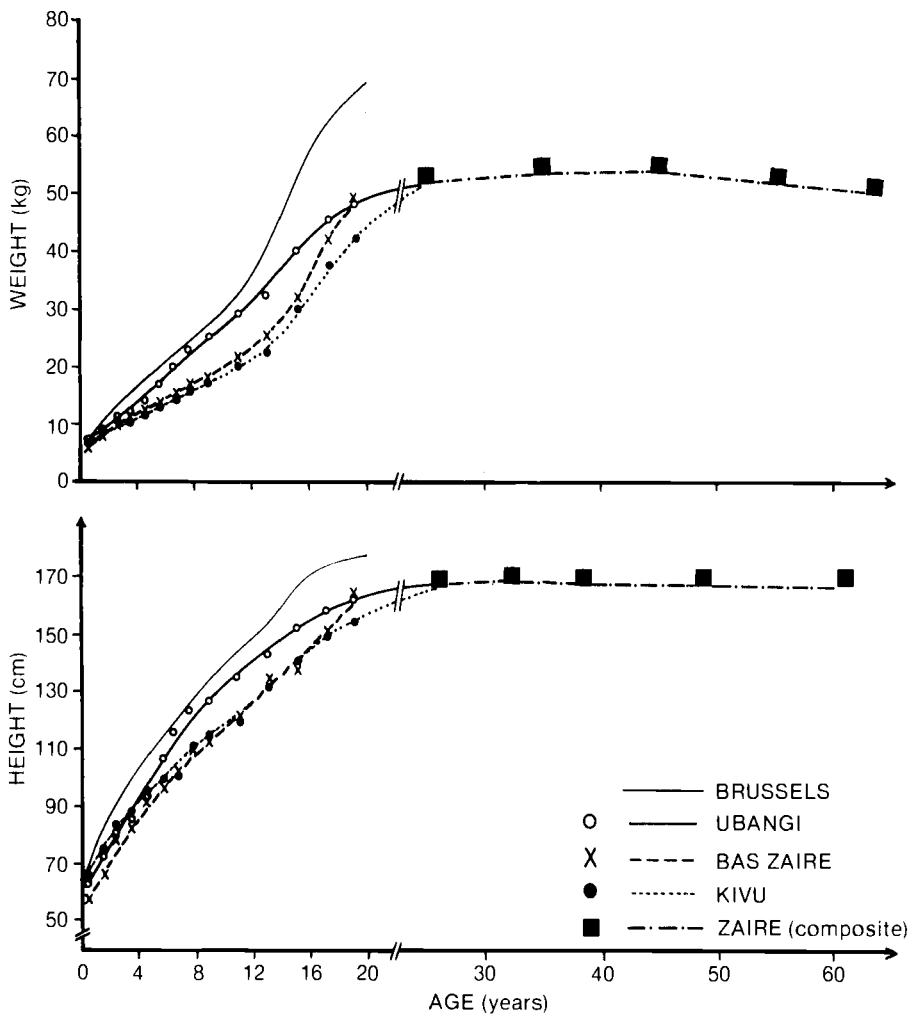
Table 4. Epidemiological data in Zaire. (Numbers of patients are shown in parentheses.)

Prevalences (%)	Bas Zaire	Kivu	Ubangi
Goitre			
Total	1.5 } (2599)	12.5 } (2687)	76.8 } (4707)
Visible	0.2 }	3.4 }	51.1 }
Nodular	0.3 }	0.7 }	18.8 }
Cretinism			
Myxedematous	0.0 } (2599)	0.0 } (2687)	4.3 } (4707)
Neurologic	0.0 }	0.0 }	0.4 }
Malnutrition (children 2–9 years)			
W-H < 80% <sup>a</sup>	16.0 (738)	26.0 (489)	8.5 (1412)
Depigmentation	0.0 } (978)	7.2 } (586)	0.7 } (275)
Edema <sup>b</sup>	0.5 }	9.0 }	1.8 }
Splenomegaly <sup>c</sup>	13.2 (725)	0.2 (429)	6.4 (501)

<sup>a</sup>Percent of children with weight-for-height below 80% of median of a standard curve.

<sup>b</sup>Of eyelids, or pretibial, or both.

<sup>c</sup>In children aged 5–14 years.



**Fig. 3.** Comparison of median weight and height as a function of age in males in Brussels, Ubangi, Bas Zaire, and Kivu.

than in Kivu but, in Bas Zaire, 16% of the children aged 2–9 years also had an abnormally low weight-for-height. Malnutrition was found only occasionally in Ubangi.

The prevalence of splenomegaly in children and adolescents aged 5–14 years was almost 15% in Bas Zaire, only 6% in Ubangi, and less than 1% in Kivu.

The development with age of weight and height in males in the three populations in Zaire, and in Belgian controls (Wachholder and Graffar 1973), is shown in Fig. 3. Weight and height were systematically lower in Zaire than in Belgium, in particular in adults. They

were similar in the three Zairian populations below the age of 2 years and above 30 years. In contrast, marked differences were observed between the three regions during late infancy, childhood, and adolescence. From the age of 2 years, the weights observed in Bas Zaire and Kivu were systematically and progressively markedly lower than in Ubangi, and in Kivu they were lower than in Bas Zaire. The maximum differences were observed at about 12 years. Subsequently, weight growth increased in Bas Zaire and Kivu so that the difference in median weight disappeared from the age of 18 years in Bas Zaire and from 25 years in Kivu.

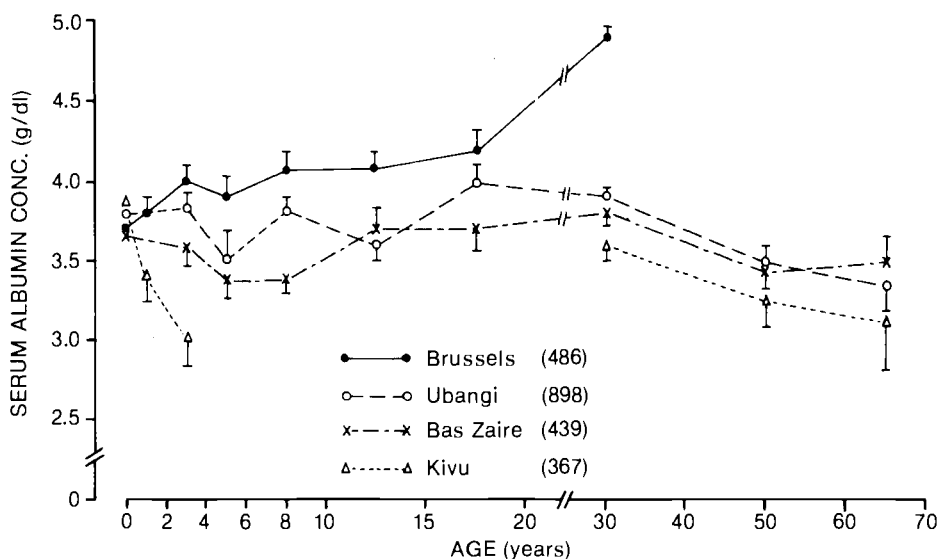


Fig. 4. Comparison of concentration of serum albumin as a function of age in Brussels, Ubangi, Bas Zaire, and Kivu. (Mean  $\pm$  SEM. Numbers of patients are given in parentheses.)

A similar phenomenon was observed for height. However, median heights did not differ between Bas Zaire and Kivu between 6 and 17 years. During the first 4 years of life, the height in Ubangi was even lower than in Kivu.

The differences observed among males in the three regions were also observed in females.

### Biochemical studies

Fig. 4 compares the changes with age of concentrations of serum albumin in Brussels and in the three rural regions in Zaire. As is further described in chapter 4, the concentrations of albumin in cord blood were similar in Brussels and in Zaire. Conversely, in patients older than 2 years, they were systematically lower in the three Zairian regions than in Brussels. The differences were more important in Bas Zaire than in Ubangi and were dramatic in the Kivu area.

A more detailed investigation of the influence of age and sex on the concentration of serum albumin was carried out in Kivu by P. Hennart and H.L. Vis (unpublished). The results are shown in Fig. 5. The serum albumin concentrations decreased drastically from 1 to 5 years. Subsequently, they increased progressively to reach a plateau at about 15 years. From 1 to 15 years, they were systematically lower in males than in females. In contrast,

during the child-bearing years, they were lower in females than in males.

Table 5 compares the urinary concentrations and daily excretions of iodine, SCN, and creatinine in Brussels, Bas Zaire, Kivu, and Ubangi. The concentrations of urinary iodine decreased progressively and those of urinary SCN increased from Brussels through Bas Zaire and Kivu to Ubangi. The results for iodine in Ubangi were 2.3 and 1.7 times lower than in Brussels and Bas Zaire respectively but were not significantly lower than in Kivu. They were lower in Kivu than in Bas Zaire ( $P < 0.001$ ). In contrast, the SCN concentrations in Ubangi were 3.0 times higher than in Brussels and 1.3 and 1.7 times higher than in Bas Zaire and Kivu. The values found in Bas Zaire and in Kivu did not differ significantly.

The creatinine concentrations were not significantly different in the four regions except in Kivu, where they were markedly lower than in Brussels, Bas Zaire, and Ubangi ( $P < 0.05-0.001$ ). In the three regions in Zaire, the coefficients of variation for creatinine and I/creatinine ratios were systematically higher than those obtained for iodine.

As a consequence of the differences in urinary iodine and SCN in the four regions, the urinary I/SCN ratios were 8.8 in Brussels, 3.8 in Bas Zaire, 2.6 in Kivu, and 1.9 in Ubangi. These differences were highly significant

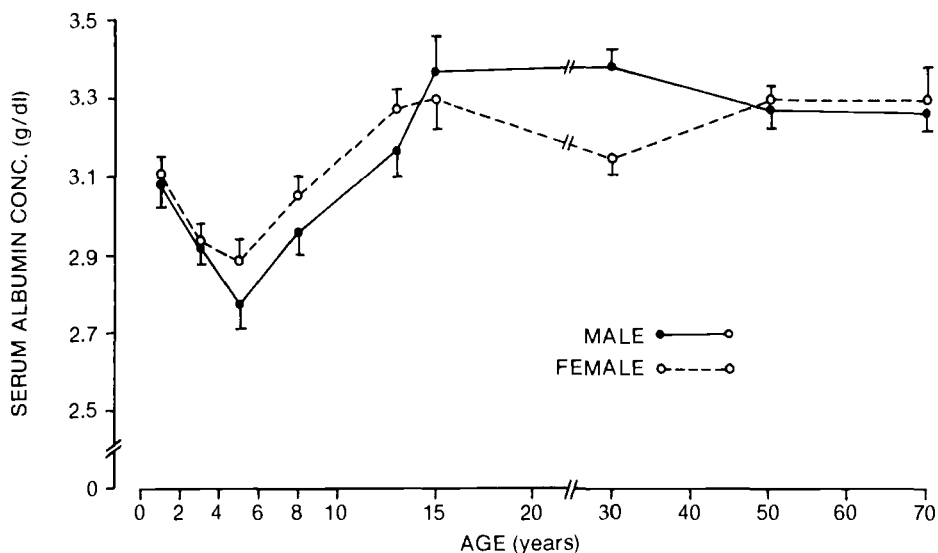


Fig. 5. Concentration of serum albumin as a function of age and sex in Kivu. (Mean  $\pm$  SEM. 1628 patients).

( $P < 0.001$ ). In contrast, the I/creatinine ratio in Kivu was 2.1 times higher than that in Ubangi and higher even than that in Brussels.

The daily urinary excretion of iodine was 51.2  $\mu\text{g/day}$  in Brussels and 15.5  $\mu\text{g/day}$  in Ubangi. Contrasting with the situation observed for the urinary iodine concentrations, the results obtained both in Bas Zaire and in Kivu were not significantly different from that for Ubangi. The daily excretions of urinary SCN were systematically higher in the three

Zairian regions than in Brussels and the highest value was found in Ubangi. Daily urinary excretion of creatinine in Kivu was half that in Brussels ( $P < 0.001$ ) and was lower than that in Bas Zaire and in Ubangi ( $P < 0.001$ ).

### Nutritional surveys

The data collected from inhabitants in the three areas in Zaire were expressed as percentages of the people questioned who had

Table 5. Comparison of the urinary concentrations and daily excretions of I, SCN, and creatinine in Brussels and in Bas Zaire, Kivu, and Ubangi. (Mean  $\pm$  SEM. <sup>a</sup> Numbers of patients are shown in parentheses.)

	Brussels (38)	Bas Zaire (24)	Kivu (157)	Ubangi (276)
Age (years)	6-20	19.8 $\pm$ 0.8**	25.5 $\pm$ 0.6***	16.5 $\pm$ 0.5 (275)
Urinary concentration				
I ( $\mu\text{g/dl}$ )	5.3 $\pm$ 0.7***	4.0 $\pm$ 0.4 (23)***	2.7 $\pm$ 0.1 (157)NS	2.3 $\pm$ 0.1 (243)
SCN (mg/dl)	0.60 $\pm$ 0.07***	1.43 $\pm$ 0.18 (24)*	1.10 $\pm$ 0.04 (156)***	1.82 $\pm$ 0.10 (140)
Creatinine (mg/dl)	115 $\pm$ 9 NS	154 $\pm$ 27 (24)NS	78 $\pm$ 4 (157)***	101 $\pm$ 4 (276)
Urinary volume (ml/day)	1088 $\pm$ 25***	540 $\pm$ 64 (23)**	597 $\pm$ 29 (153)***	745 $\pm$ 26 (276)
Daily urinary excretion				
I ( $\mu\text{g}$ )	51.2 $\pm$ 5.8***	20.5 $\pm$ 2.9 (22)NS	14.7 $\pm$ 1.0 (153)NS	15.5 $\pm$ 1.3 (243)
SCN (mg)	5.37 $\pm$ 1.07***	7.24 $\pm$ 1.09 (24)*	5.88 $\pm$ 0.31 (152)***	10.75 $\pm$ 0.61 (140)
Creatinine (mg)	809 $\pm$ 103***	744 $\pm$ 160 (23)NS	401 $\pm$ 21 (153)***	609 $\pm$ 18 (276)
Urinary ratios				
I/creatinine ( $\mu\text{g/g}$ )	54 $\pm$ 10***	42 $\pm$ 10 (23)NS	60 $\pm$ 8 (157)***	29 $\pm$ 2 (243)
SCN/creatinine (mg/g)	7 $\pm$ 1***	12 $\pm$ 1 (24)***	26 $\pm$ 4 (156)NS	20 $\pm$ 2 (140)
I/SCN ( $\mu\text{g/mg}$ )	8.8 $\pm$ 0.4***	3.8 $\pm$ 0.7 (23)***	2.6 $\pm$ 0.12 (156)**	1.9 $\pm$ 0.21 (121)

<sup>a</sup>Levels of significance refer to the comparison with the results from Ubangi: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Table 6. Percentages of inhabitants questioned who had consumed the principal food items within the previous 24 hours.

Food items	Ubangi	Kivu	Bas Zaire
Cassava gruel	90	-	-
Cassava leaves	63	29	56
<i>Chickwangue</i>	7	-	40
Boiled cassava roots	9	3	20
Cassava paste	-	62	80
Cassava paste + sorghum flour	-	16	-
Grilled roots	-	-	12
Maize	28	1	8
Rice	3	3	12
Bananas	6	10	-
Peanuts	21	2	16
Yams	-	1	20
Green leaves	-	17	32
Fruits	-	1	40
Beans	-	48	-
Sorghum	-	2	-
Fish	16	36	32
Meat	11	20	8
Other animal proteins	18	3	-

consumed a given food at least once during the previous 24 hours. The results for the main food items are shown in Table 6. The local names of the food items and their methods of preparation are described in chapter 5.

In Ubangi, cassava gruel (*fuku*) was by far the food of choice (90%). Cassava leaves (*mpondu*: 63%), frequently accompanying *fuku*, was considered to be another attractive food-stuff. With *fuku* and *mpondu*, boiled sweet roots (*nsongo*: 9%) and *chickwangue* (*kwanga*: 7%) represented the various forms in which cassava was eaten during meals. Besides the cassava products, other sources of carbohydrates were maize (28%), bananas (6%), and rice (3%). Yams and sweet potatoes were only eaten by a negligible part of the population surveyed. Peanuts (21%) accounted for an appreciable intake of fatty acids. In addition to proteins from vegetables, the main sources of animal proteins were meat (11%), fish (16%), and insects (18%).

In Bas Zaire, the most popular foodstuffs were cassava paste (*fufu*: 80%) and cassava leaves (*nsaki*: 56%). However, *chickwangue* (*nsua*, *ntinga*, or *nsesa*: 40%), boiled roots (*mateloko*: 20%), and grilled roots (*bikedi*: 12%) were eaten in larger amounts than in Ubangi. Compared to Ubangi, maize consumption was

lower (8%) whereas rice consumption was higher (12%). Other vegetables, mainly green leaves, were eaten frequently (32%). For protein intake, the situation was rather different from Ubangi, meat consumption (8%) was about the same but fish consumption was twice as frequent (32%).

In Kivu, cassava products were mainly consumed as cassava paste (*bugali*: 62%), cassava leaves (*sombe*: 29%), and cassava paste supplemented with sorghum flour (*bugali ya mohogo na mutama*: 16%); boiled roots were an insignificant food item (3%). Contrasting with Ubangi and Bas Zaire, inhabitants of Kivu frequently consumed beans (48%), which represented the alternate source of carbohydrate. Bananas (10%) and green leaves (17%) were occasionally mentioned by the persons surveyed. Consumption of fish, mainly small fry, was similar (36%) to that reported for Bas Zaire.

In the three areas, sugarcane and raw cassava roots (mainly sweet) were the food items most frequently consumed between meals. In Bas Zaire, fruits were also included in this category.

Because our focus was the role played by cassava, we calculated the number of subjects who had consumed cassava at least once during the 24 hours preceding the interview. The results for this estimate were over 90% in all three areas — Bas Zaire, 96%; Kivu, 93%; and Ubangi, 91%.

These values show that cassava was widely used and represented the staple food of the populations in the three areas. We should point out, however, that the nutritional survey in Kivu was carried out in July and August, i.e., the period when cassava was harvested. From December to June, when cassava was not eaten, Kivu inhabitants consume larger amounts of beans, yams and sweet potatoes.

## Discussion

These findings confirm the existence of marked regional variations in the prevalence of goitre and malnutrition and in the dietary supply of iodine, SCN, and proteins in Bas Zaire, Kivu, and Ubangi. They show that the dietary supply of iodine plays a crucial role in the development of goitre in the presence of a cassava-based diet while even extreme pro-

tein calorie malnutrition in humans apparently does not seem to critically impair the endogenous conversion to SCN of HCN released from cassava.

The epidemiological data on the prevalence of endemic goitre and cretinism in Ubangi and on malnutrition in Kivu confirm previous more extensive studies carried out in the same areas (Vis 1963; Ermans et al. 1980a). The prevalence of goitre of 12.5% for Kivu in the present study is seven times higher than the figure of 1.8% reported in 1972 by Thilly et al. from exactly the same villages. This development confirms the clinical descriptions of the CEMUBAC-IRS team of nutritionists who have worked in the area for nearly 20 years and indicates that, according to the criteria of Querido et al. (1974), this part of Kivu is now affected by mild endemic goitre. Our work shows that prevalence of goitre in Bas Zaire is not abnormal but that the protein calorie nutritional status of this population is more critical than in Ubangi, as reflected by a more important delay in weight and height growth, and by lower concentrations of serum albumin. These observations confirm data recently collected in other parts of Bas Zaire (N. Kabamba, R. Franklin, et al. unpublished). Malnutrition in Bas Zaire could be aggravated by chronic infections, such as malaria, as suggested by the high prevalence of splenomegaly in this region.

This work shows that the dietary supply of iodine is only barely lower in Bas Zaire than in Brussels, but is markedly decreased in both Kivu and Ubangi. The subnormal value found in Bas Zaire for iodine supply could be explained by the relative proximity of the ocean and by a frequent consumption of fish. The values for Kivu are close to those reported from the same area in 1972 (Thilly et al.) and are similar to those for Ubangi. This conclusion is also supported by more extensive studies reported in chapter 3.

These data clearly show that neither the increase in the prevalence of goitre observed in Kivu during the last 8 years nor the difference in prevalence of goitre between Kivu and Ubangi can be explained by differences in the iodine supply.

The concentrations and excretions of urinary SCN are markedly higher in the three regions in Zaire than in Brussels and also in Ubangi than either Bas Zaire or Kivu, where they are rather similar. Consequently, the urinary I/SCN ratio decreases progressively from Brussels through Bas Zaire and Kivu to Ubangi.

Endemic goitre is not found as long as this ratio is higher than a critical threshold of 3–4 and is hyperendemic (Ubangi) only when this ratio is below 2. These values are identical to those reported for endemic goitre in Sicily (Delange et al. 1978) and to those reported in chapters 3 and 7.

This study shows that cassava constitutes one of the major staple foods in the three rural areas investigated in Zaire and strongly suggests that the SCN overload observed in these areas results from the intake of cassava. However, our nutritional surveys were only qualitative and did not account for seasonal variations in the intake of cassava, which are important, particularly in Kivu (Vis et al. 1969). Consequently, the present data do not allow us to establish a direct relationship between the quantity of cassava ingested and the degree of SCN overload. Moreover, the results in chapter 5 show that the HCN content of fresh cassava roots and the detoxification processes for fresh cassava differ markedly among the three areas. These differences also influence the quantity of HCN ingested from cassava.

No figures were available for urinary or serum SCN in Kivu when Thilly et al. (1972) surveyed goitre, but the results of our nutritional survey in this area show that cassava is now a staple food whereas an extensive quantitative nutritional survey in the same area in 1965 and 1966 showed that it represented only 17.6% of the annual mean calorie intake (Vis et al. 1969). This modification of food habits is one of the results of the dramatic food shortage occurring in the area.

Our data strongly suggest that the recent development of endemic goitre in Kivu is a direct consequence of increasing intake of cassava in the presence of an unchanged degree of iodine deficiency.

In contrast, the data collected in Bas Zaire clearly indicate that chronic consumption of large quantities of cassava-based foods does not necessarily result in a dramatic SCN overload nor in endemic goitre (see also chapter 5).

In spite of extreme clinical and biochemical signs of malnutrition observed in Kivu, endogenous conversion of HCN to SCN is still possible, suggesting that the sulfur amino acids required for this conversion are still at least partially available. This hypothesis is confirmed by the results reported in chapter 8.

The possibility arises that the prevalence of goitre in Kivu is lower than would be expected



on the basis of I/SCN ratio because of concomitant malnutrition.

It appears that the concentrations of urinary iodine and SCN measured on casual urine samples constitute the most accurate index of the iodine and SCN supplies provided that the number of samples is large enough to compensate for individual variability for the following two reasons.

- Urinary creatinine is much lower in Kivu than in Brussels, Bas Zaire, and Ubangi as a consequence of malnutrition. Consequently, the I/creatinine ratio in Kivu is higher than in the other areas, including Brussels, in spite of very low iodine concentrations. The value of 60 found in Kivu for this ratio is within the normal range according to the criteria proposed by Querido et al. (1974). This shows that the I/creatinine ratio is not accurate to assess iodine intake in the presence of malnutrition and that this ratio should be discarded as a universal index of the iodine supply. Moreover, our data show that the use of the urinary I/creatinine ratio as an index of iodine intake, which is supposed to reduce the interindividual variability as compared to the urinary concentration of iodine, actually increases this variability, even in the absence of malnutrition.

- The daily urinary excretions of iodine and SCN in the three Zairian regions are less different than their concentrations in casual samples, in particular for iodine in Bas Zaire. This situation could be critically influenced by the accuracy of the daily collections of urine, which could have been incomplete in Bas Zaire.

For these two reasons, in the next stages of this work, we considered only the concentrations of iodine and SCN in the urine and not their ratios to creatinine nor their daily excretions.

In conclusion, this work shows that, in the presence of a cassava-based diet and of a low iodine supply, as observed in the three rural

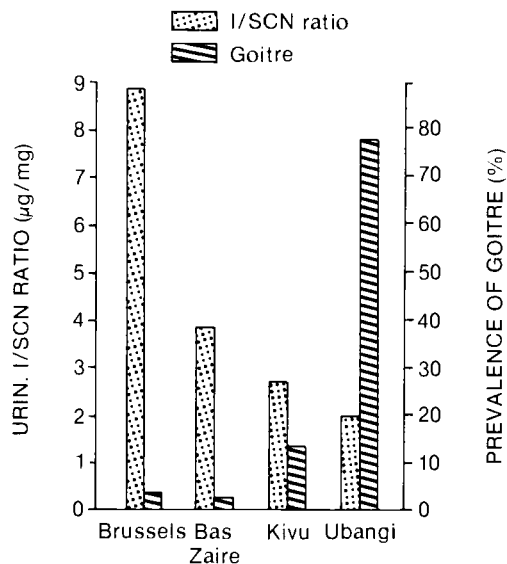


Fig. 6. Relationship between the urinary I/SCN ratio and the prevalence of goitre.

regions investigated in Zaire, marked differences in the prevalence of goitre are not explained solely by differences in the iodine supply. As shown in Fig. 6, in these conditions, the development of goitre is critically dependent on the dietary balance between iodine and SCN. This balance is most clearly shown by the determination of urinary I/SCN ratios in representative samples of the population. Under normal conditions, this ratio is higher than 7. Goitre appears when the ratio reaches a critical threshold of about 3 and becomes hyperendemic and complicated by cretinism only when it is lower than 2.

Thus, a cassava-based diet does not necessarily result in the development of goitre provided that the iodine intake is high enough or that the HCN content of foods prepared from cassava is low enough. Moreover, this work shows that the massive introduction of cassava in the presence of iodine deficiency may result in the development of endemic goitre in previously unaffected populations.



### Chapter 3

## Influence of the Dietary Balance of Iodine/ Thiocyanate and Protein on Thyroid Function in Adults and Young Infants

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**Influence de la balance alimentaire en iode/thiocyanate et de l'apport protéique sur la fonction thyroïdienne de l'adulte et du jeune enfant — Résumé —** Le présent travail a eu pour objet d'apprécier l'influence des conditions nutritionnelles d'environnement sur la fonction thyroïdienne de l'adulte au Bas-Zaïre, au Kivu et en Ubangi et de comparer dans chacune de ces trois régions la fonction thyroïdienne de l'enfant âgé de 1 à 3 ans à celle de l'adulte. Des patients belges normaux ont été utilisés comme témoins.

Parallèlement à une diminution progressive du rapport urinaire I/SCN de Bruxelles (8,8), au Bas-Zaïre (3,7), au Kivu (3,0) à l'Ubangi (1,6) déjà rapportée antérieurement sur un nombre de patients plus limité (chapitre 2), on observe chez

l'adulte au Zaïre par rapport aux contrôles belges (Tableau 7) une diminution progressive et significative des taux de  $T_4$  du Bas-Zaïre, au Kivu, à l'Ubangi. Les taux de TSH ne sont toutefois augmentés que dans la dernière région. Les taux de  $T_3$  sont normaux au Bas-Zaïre, abaissés au Kivu et augmentés en Ubangi. En dépit de concentrations urinaires d'iode identiques et plus basses respectivement au Bas-Zaïre et au Kivu qu'à Bruxelles, la captation thyroïdienne de radioiode est par rapport à celle du groupe témoin, plus basse dans la première région et non significativement différente dans la seconde mais beaucoup plus élevée en Ubangi.

Les différences observées entre les enfants des trois régions investiguées au Zaïre et les témoins belges du même âge (Tableau 8) sont assez similaires à celles observées chez l'adulte. De plus, les enfants de l'Ubangi ont des taux de TSH beaucoup plus élevés que les adultes de la même région en dépit du fait qu'ils sont soumis au même degré de carence iodée et qu'ils ont des taux de  $T_4$  plus élevés (Tableau 8, Fig. 7).

En conclusion, une diminution de la balance entre les apports alimentaires en iode et en SCN va de pair chez l'adulte et l'enfant de 1 à 3 ans avec un abaissement progressif de la  $T_4$  sérique. Une augmentation de la TSH n'est cependant observée que pour un rapport I/SCN urinaire d'environ 2, soit un taux plus bas que celui qui conditionne l'apparition de goitre. Les observations rapportées indiquent que l'enfant est plus sensible que l'adulte à l'action antithyroïdienne combinée de la carence iodée et de la surcharge en SCN résultant de la consommation de manioc.

Il n'est pas exclu que la malnutrition existante au Bas-Zaïre et au Kivu soit partiellement responsable des taux de  $T_4$  abaissés et des captations plus basses que celles attendues sur la base de l'apport iodé dans ces deux régions. La surcharge en SCN pourrait également expliquer cette dernière observation.

The results in chapter 2 show that the three rural populations in Zaire suffer varying degrees of iodine deficiency and of SCN overload resulting in progressively decreasing urinary I/SCN ratios from Bas Zaire, through Kivu, to Ubangi. They also show that protein calorie malnutrition is hyperendemic only in Kivu, although the protein supply in Bas Zaire is also clearly insufficient.

Thus the question arises "to what extent do these nutritional characteristics of the diet influence thyroid function in the affected populations?" The influence of dietary environmental factors on thyroid function in a given

population is usually studied in adults (review by Delange and Ermans 1976). However, studies reported previously from the Idjwi island endemic goitre area have shown that the metabolic picture observed in adults only represents the final stage of a process of adaptation that is critically influenced by age (Delange 1974). Moreover, alterations of thyroid function during the first 3 years of life in humans are particularly important because they occur during the critical period of brain development (Dobbing and Sands 1973; Dobbing 1974) and may result in irreversible brain damage and mental retardation (review by Delange 1981). Finally, it has been observed previously that, in Ubangi, adults had markedly higher serum SCN concentrations than infants (Delange et al. 1980a).

The purposes of the investigations reported in this chapter have been:

- To compare thyroid function and variables exploring the dietary supplies of iodine, SCN, and proteins in adults in Bas Zaire, Kivu, and Ubangi with Brussels as a control region.
- To make the same comparison in infants aged 1–3 years.
- To study the evolution of concentration of serum SCN as a function of age in Ubangi.

## Patients and Methods

The investigation was carried out in randomly selected, clinically euthyroid patients aged 0.5–65 years in Bas Zaire, Kivu, and Ubangi. No metabolic studies could be performed in the general population of Kinshasa. Particular attention was paid to young adults aged 15–39 years and to infants aged 1–3 years. The patients were selected without regard to the presence or absence of goitre. The results in Zaire were compared with those in normal Belgian patients of the same age used as controls. The present investigations also included a group of myxedematous endemic cretins of the Ubangi area in whom the diagnosis was based on obvious clinical evidence of severe mental retardation and hypothyroidism (Delange et al. 1972c). The numbers of patients investigated in Brussels, Bas Zaire, Kivu, and Ubangi are shown in Tables 7 and 8 and in Fig. 7.

Thyroid function was assessed by determining the thyroïdal uptake of radioiodine at 6 and 24 hours and the concentrations of serum TSH,  $T_4$ , and  $T_3$ .

The dietary supplies of iodine and SCN were estimated from their urinary concentrations and the urinary I/SCN ratio, and the supply of proteins from the concentrations of serum total proteins and albumin and of urinary creatinine.

## Results

The results obtained in clinically euthyroid adults are shown in Table 7 with those for myxedematous cretins in Ubangi. Compared to the results obtained in the Belgian controls, the following differences were observed in clinically euthyroid adults in Zaire:

- The serum and urinary concentrations of SCN were significantly higher in the three areas; serum SCN concentrations were increased by a factor 3 in both Bas Zaire and Kivu and by a factor 4 in Ubangi. The results obtained in these two first regions were not significantly different.
- The urinary concentrations of iodine were not significantly different in Bas Zaire but were markedly lower in Kivu and Ubangi. The value of 2.1  $\mu\text{g}/\text{dl}$  for Ubangi was still lower than the corresponding value of 3.7 for Kivu ( $P < 0.001$ ). Consequently, the urinary I/SCN ratios were 8.8 in Brussels, 3.7 in Bas Zaire, 3.0 in Kivu, and 1.6 in Ubangi. They were significantly lower in Ubangi than in Kivu ( $P < 0.001$ ) and in Kivu than in Bas Zaire ( $P < 0.01$ ).
- Thyroïdal uptake of radioiodine was lower in Bas Zaire, not significantly different in Kivu, and markedly higher in Ubangi.
- Serum  $T_4$  was slightly lower in Bas Zaire and markedly lower in Kivu and Ubangi.
- Serum  $T_3$  was not significantly different in Bas Zaire but was lower in Kivu and higher in Ubangi.
- Serum TSH was not significantly different in Bas Zaire and Kivu but was markedly higher in Ubangi where the mean value was 10 times higher than for the controls.
- Total proteins were not significantly different in Bas Zaire but were lower in Kivu and higher in Ubangi.
- In contrast, albumin was significantly lower in the three areas investigated in Zaire. The lowest value, in Kivu, was 1.3 times lower than in Brussels.
- The concentration of urinary creatinine was slightly higher in Bas Zaire and Ubangi but was markedly lower in Kivu. Therefore, the I/creatinine ratio was not significantly different in Bas Zaire and Kivu than in Brussels while it was markedly lower in Ubangi.

Table 7. Comparison of the results obtained for the variables exploring thyroid function and dietary supplies of iodine, SCN, and proteins in clinically euthyroid adults aged 15–39 years in Brussels, Bas Zaire, Kivu, and Ubangi and in myxedematous endemic cretins in Ubangi. (Mean  $\pm$  SEM. <sup>a</sup> Numbers of patients are shown in parentheses.)

Variables	Brussels	Bas Zaire	Kivu	Ubangi	
				Clinically euthyroid	Myxedematous cretins
Serum concentration					
SCN (mg/dl)	0.26±0.01 (113)	0.80±0.04*** (87)	0.74±0.05*** (118)	1.05±0.04*** (292)	1.61±0.12*** (76)
T <sub>4</sub> (μg/dl)	8.1±0.1 (125)	7.7±0.2* (186)	5.2±0.1*** (176)	4.9±0.2*** (358)	0.50±0.01*** (120)
T <sub>3</sub> (ng/dl)	144±3 (124)	150±3NS (90)	130±3** (178)	166±3*** (299)	46±3*** (109)
TSH (μU/ml)	1.7±0.1 (125)	2.7±0.2*** (183)	1.7±0.1NS (178)	18.6±2.1*** (365)	302.7±20.0*** (122)
Proteins (g/dl)	7.82±0.07 (117)	7.61±0.12NS (168)	7.04±0.10*** (141)	8.19±0.06*** (294)	7.57±0.12NS (99)
Albumin (g/dl)	4.87±0.04 (117)	3.75±0.07*** (170)	3.65±0.07*** (144)	3.99±0.04*** (303)	3.41±0.07*** (99)
Urinary concentration					
I (μg/dl)	5.3±0.7 (38)	5.6±0.3NS (151)	3.7±0.2*** (245)	2.1±0.1*** (374)	1.0±0.2*** (19)
SCN (mg/dl)	0.60±0.07 (38)	1.88±0.08*** (176)	1.55±0.09*** (252)	2.18±0.10*** (428)	2.04±0.24*** (43)
Creatinine (mg/dl)	115±9 (38)	154±28* (23)	86±5*** (111)	148±5* (338)	65±6*** (38)
Urinary ratio					
I/SCN (μg/mg)	8.8±0.4 (38)	3.7±0.2*** (145)	3.0±0.1*** (244)	1.6±0.1*** (370)	1.1±0.2*** (19)
I/creatinine (μg/g)	54±10 (38)	43±11NS (22)	56±9NS (111)	21±2*** (285)	14±2*** (19)
<sup>131</sup> I Thyroidal uptake (% dose)					
6-hour	–	18.0±0.9 (58)	24.0±1.9 (48)	53.2±1.5 (123)	–
24-hour	46.4±1.1 (255)	33.1±1.2*** (63)	41.8±2.3NS (45)	65.2±0.9*** (167)	28.3±2.6*** (6)

<sup>a</sup>Levels of significance refer to the comparison with the results from Brussels: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

Compared to the results obtained in clinically euthyroid adults in Ubangi, myxedematous cretins had extremely markedly higher TSH and lower T<sub>4</sub> and T<sub>3</sub> concentrations and thyroidal uptake of radioiodine. They also had higher serum SCN, and lower serum proteins and albumin and urinary iodine.

The corresponding results obtained in infants aged 1–3 years are shown in Table 8. In Brussels, as in the three regions investigated in Zaire, T<sub>4</sub> was systematically higher in infants than in adults. The differences observed between the Zairian groups and the Belgian controls were rather similar to those observed in adults. In addition, infants in Ubangi had notably higher TSH than adults in spite of the fact that they had higher T<sub>4</sub>.

The changes with age in concentrations of serum SCN and of urinary iodine in patients from Brussels and Ubangi are shown in Fig. 7. In the control group (Brussels), the mean concentration of serum SCN remained fairly constant from birth to adulthood and was close to 0.30 mg/dl. In contrast, in Ubangi, this concentration decreased progressively from birth to 5 years and then markedly and constantly increased. It reached a mean value of 1.12 mg/dl at 30 years and 1.30 mg/dl at 50 years.

The urinary concentration of iodine in Ubangi did not show any significant modification with age and remained close to 2  $\mu$ g/dl. It was 1.6–2.5 times lower than in the four age groups of Belgian controls for which data were available (P < 0.001).

Table 8. Comparison of concentrations of serum SCN, thyroid hormones, TSH, proteins, and albumin in infants aged 1–3 years in Brussels and Zaire. (Mean  $\pm$  SEM. <sup>a</sup>Numbers of patients are shown in parentheses.)

Variables	Brussels	Bas Zaire	Kivu	Ubangi
SCN (mg/dl)	0.32 $\pm$ 0.03 (34)	–	–	0.49 $\pm$ 0.03*** (91)
T <sub>4</sub> ( $\mu$ g/dl)	9.9 $\pm$ 0.2 (96)	10.2 $\pm$ 0.5NS (40)	8.1 $\pm$ 0.5*** (43)	7.7 $\pm$ 0.4*** (131)
T <sub>3</sub> (ng/dl)	179 $\pm$ 5 (91)	–	183 $\pm$ 13NS (21)	177 $\pm$ 7NS (71)
TSH ( $\mu$ U/ml)	2.2 $\pm$ 0.2 (94)	3.1 $\pm$ 0.3* (41)	1.1 $\pm$ 0.1*** (64)	40.2 $\pm$ 9.5*** (129)
Proteins (g/dl)	6.35 $\pm$ 0.12 (68)	6.49 $\pm$ 0.12NS (46)	6.42 $\pm$ 0.23NS (13)	6.74 $\pm$ 0.08** (134)
Albumin (g/dl)	3.89 $\pm$ 0.08 (68)	3.70 $\pm$ 0.08NS (46)	3.34 $\pm$ 0.13*** (22)	4.23 $\pm$ 0.06*** (125)

<sup>a</sup>Levels of significance refer to the comparison with the results from Brussels: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

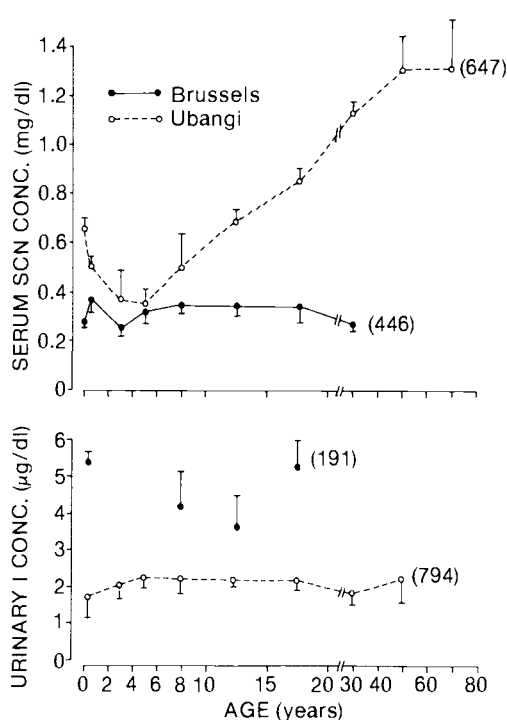


Fig. 7. Comparison of serum SCN and urinary iodine concentrations as a function of age in Brussels and Ubangi. (Mean  $\pm$  SEM. Numbers of patients are shown in parentheses.)

## Discussion

This study shows that, in populations experiencing varying degrees of iodine deficiency and SCN overload, thyroid function is

clearly impaired in adults only when the urinary I/SCN ratio is lower than a critical threshold of about 2. It also shows that the biochemical indices in such an environment differ markedly between adults and infants.

The biochemical profiles observed in clinically euthyroid adults and in myxedematous endemic cretins in Ubangi are similar to those reported earlier for Kivu (Delange et al. 1971, 1972b; Thilly et al. 1972) and for Ubangi (Lagasse et al. 1980a, c). Compared to Belgian controls, euthyroid adults have markedly higher concentrations of serum and urinary SCN, lower urinary iodine, and consequently markedly lower urinary I/SCN ratio. Their mean concentrations of serum TSH and T<sub>4</sub> would be highly suggestive of hypothyroidism in Western countries. However, their T<sub>3</sub> concentrations are higher than in the controls. As discussed previously for the Idjwi endemic goitre area (Delange et al. 1972a), this adaptive mechanism could partly account for the absence of clinical signs of overt hypothyroidism in these patients.

Compared to clinically euthyroid adults of the Ubangi area, myxedematous endemic cretins have much higher TSH values and much lower values of T<sub>4</sub>, T<sub>3</sub>, and thyroidal uptake, confirming the presence of an extremely severe thyroid failure. They also have higher levels of serum SCN and lower serum proteins and albumin, and urinary iodine and creatinine. These latter findings suggest that the nutritional status of these patients is less adequate than that of the general population. The present data do not allow us to establish whether this situation is the cause or the consequence of their thyroid failure.

In spite of urinary I/SCN ratios that are 2.4 and 2.9 times lower in Bas Zaire and Kivu than in Brussels, adults in these two Zairian regions have normal or even low TSH, strongly suggesting that the supply of thyroid hormones to peripheral tissues, including the pituitary, is adequate. However, total  $T_4$  is slightly decreased in Bas Zaire but markedly decreased in Kivu while  $T_3$  is normal in the former region and decreased in the latter. This particular biochemical profile most probably results at least partly from protein malnutrition, which is clearly demonstrated by decreased concentrations of total proteins and, mainly, albumin. In malnutrition, low total  $T_4$  and  $T_3$  values are due both to decreased concentrations of thyroid hormone-carrier proteins, in particular TBPA, and to a shift of the peripheral monodeiodination of  $T_4$  to  $rT_3$  instead of  $T_3$  resulting in a low  $T_3$  syndrome (review by Ingenbleek 1980). The absence of elevated TSH in this condition is explained by the fact that the free fractions of  $T_4$  and  $T_3$  are usually normal (Chopra and Smith 1975). The concentrations of free  $T_4$ , free  $T_3$ ,  $rT_3$ , and of the thyroid hormone-carrier proteins, TBG and TBPA, were not determined in our study. As a result, the state of biochemical euthyroidism and the respective roles played by the carrier proteins and by the shift of monodeiodination in the pathogenesis of the low  $T_3$  syndrome in these patients could not be further assessed.

The observation that thyroïdal uptake of radioiodine is similar in Kivu and Brussels, despite the lower iodine supply in the former region, could possibly be explained by SCN overload in Kivu as this inhibits trapping of iodide by the thyroid (Delange and Ermans 1971). The same explanation could perhaps explain the results for Bas Zaire where the urinary iodine value is similar to that for Brussels but where thyroïdal uptake is lower. We cannot exclude the possibility that the relatively low thyroïdal uptakes in Bas Zaire and Kivu could also be partly explained by the state of malnutrition (El-Gholmy et al. 1967; Ingenbleek and Beckers 1977).

The biochemical patterns found in Zairian infants aged 1–3 years and in adults living in the same environmental conditions differ strikingly. In Belgium, infants aged 1–3 years have TSH values that are similar to those in adults, but  $T_4$  and  $T_3$  values that are slightly higher. This classical finding (Ryness 1972; Dutau et al. 1974; Roger et al. 1975; Corcoran et al. 1977; Fisher et al. 1977a; Garcia-Bulnes

et al. 1977) results from higher TBG concentrations in infants than in adults (Fisher et al. 1977a; Hesch et al. 1977; Stubbe et al. 1978; Parra et al. 1980), although free  $T_4$  values are similar in the two age groups (Hays and Mullard 1974; Fisher et al. 1977a). Similar differences between infants and adults are observed in Bas Zaire and Kivu, although the differences for  $T_4$ , particularly in Kivu, are more important than in the Belgian controls. As stated above, the interpretation of this situation is difficult in the absence of results for free  $T_4$  and the carrier proteins.

The most striking difference between infants and adults is observed in Ubangi. Here again, infants have higher  $T_4$ , and to a lesser extent  $T_3$ , values than adults; and yet they also have markedly higher TSH. The mean value of 40  $\mu$ U/ml for TSH in this age group would indicate overt hypothyroidism according to the criteria used in the Western countries (Delange et al. 1980c). The biochemical picture observed in infants in Ubangi is at variance with the one previously reported from the Idjwi island endemic goitre area where PBI was identical in infants and adults, or even slightly lower (Delange 1974), and where TSH was not significantly changed with age (Delange et al. 1971). These discrepancies are possibly related to methodology.

The data for Ubangi therefore indicate that, in severe endemic goitre, significant TSH hyperstimulation is required during infancy to maintain a subnormal concentration of circulating thyroid hormones, probably because iodine stores of the thyroid are markedly lower (Wilkin et al. 1977) in infants than in adults as has been previously demonstrated in endemic goitre (Delange 1974). This biochemical picture of subnormal  $T_4$  and markedly elevated TSH in infants corresponds to a state of subclinical hypothyroidism (Evered et al. 1973) as observed in Western countries in infants with small ectopic residues of thyroid tissue (Delange et al. 1980c).

Thus, the data support the concept that, in severe endemic goitre, infants are more at risk of overt thyroid failure than adults (Delange 1974). This point is more extensively discussed in chapter 6.

The change in the concentration of serum SCN during growth in Ubangi most probably reflects important differences in food habits as a function of age: the progressive decrease starting from birth to 5 years of age, analyzed in more detail in chapter 6, is closely related to

breast feeding. The subsequent marked increase in serum SCN could reflect progressive increases in cassava consumption, which is maximal in the less active part of the population, such as old people, and cretins.

In conclusion, thyroid function in populations with different levels of dietary supply of iodine, cassava, and proteins is critically related to the balance between the dietary supplies of iodine and SCN, which is most accurately determined by the urinary I/SCN ratio. A decrease in this ratio is accompanied by a progressive decrease of  $T_4$ . Significant hypersecretion of TSH by the pituitary is observed only when the ratio is lower than a critical level of about 2. This threshold is not reached, even in conditions of very high SCN overload, unless the iodine supply is concomitantly markedly decreased. This critical level is lower than the value of 3-4 corresponding

to the development of endemic goitre in the population (see chapter 2). This observation agrees with previous studies reporting that TSH is not increased in adults in moderate endemic goitre (Coble and Kohler 1970; Pisarev et al. 1970; Horn et al. 1972; Pinchera et al. 1973).

The observation that infants aged 1-3 years have much higher TSH than adults, in spite of a similar degree of iodine deficiency, indicates that this age group is more sensitive than adults to the antithyroid action of combined iodine deficiency and SCN overload resulting from ingestion of cassava.

Finally, the low levels of serum  $T_4$  observed in Bas Zaire and Kivu could also be explained, at least partly, by the presence of protein malnutrition. As stated above, however, the data now available do not allow us to analyze this possibility.



#### Chapter 4

### Influence of Dietary Goitrogens during Pregnancy in Humans on Thyroid Function of the Newborn

F. DELANGE, C. THILLY, P. BOURDOUX, P. HENNART, P. COURTOIS, AND A.M. ERMANS

**Influence de goitrigènes alimentaires durant la grossesse sur la fonction thyroïdienne du nouveau-né — Résumé —** Le présent travail a eu comme objectifs : 1) d'apprécier l'influence de l'apport alimentaire en iode et en SCN chez la femme enceinte sur la fonction thyroïdienne du nouveau-né; 2) de préciser le rôle joué par le SCN d'origine maternelle sur cette fonction; et 3) de comparer les relations existant entre les fonctions thyroïdiennes maternelle et foetale dans les conditions physiologiques et en présence de goitrigènes alimentaires.

Les concentrations sériques de TSH,  $T_4$ ,  $T_3$  et SCN ont été déterminées sur le sang du cordon chez 674 nouveau-nés étudiés à Bruxelles, Kinshasa, au Bas-Zaïre, au Kivu et en Ubangi et chez 637 des mères au moment de l'accouchement. Les concentrations urinaires d'iode et de SCN et les rapports urinaires I/SCN ont également été déterminés chez les mères. La maternité de Kinshasa a été utilisée comme contrôle.

Comme chez les adultes des trois régions rurales investiguées au Zaïre, on observe chez les femmes à l'accouchement une augmentation progressive des concentrations sériques et urinaires de SCN et une diminution des rapports urinaires I/SCN en passant de Kinshasa au Bas-Zaïre au Kivu et en Ubangi (Tableau 9). Comme pour la population adulte également, une augmentation appréciable de la TSH sérique par rapport aux contrôles n'est toutefois observée chez la femme enceinte qu'en Ubangi (15,8  $\mu\text{U/ml}$  versus 3,4  $\mu\text{U/ml}$ ,  $P < 0,001$ ). Par contre, la TSH au cordon est déjà plus élevée au Bas-Zaïre (9,8  $\mu\text{U/ml}$ ) et au Kivu (10,1  $\mu\text{U/ml}$ ) que chez les contrôles (7,8  $\mu\text{U/ml}$ ,  $P < 0,01$ ) et, en Ubangi, elle est augmentée par un facteur 10 (69,8  $\mu\text{U/ml}$ ,  $P < 0,001$ ) (Fig. 8). Dans cette dernière région, 11,2 % des nouveau-nés présentent un tableau biochimique d'hypothyroïdie congénitale sévère caractérisé par l'association sur le sang de cordon de taux de TSH supérieurs à 100  $\mu\text{U/ml}$  et de  $T_4$  inférieurs à 3  $\mu\text{g/dl}$  (Fig. 9).

Il a été clairement confirmé que dans l'espèce humaine, le SCN franchit librement la barrière placentaire (Fig. 10). Le rôle joué par la surcharge en SCN d'origine maternelle dans l'altération de la fonction thyroïdienne du nouveau-né au Zaïre est attesté par le fait qu'en Ubangi, où le degré de carence iodée des mères est identique à celui observé au Kivu, une surcharge maternelle en SCN plus importante va de pair avec des altérations extrêmes de la fonction thyroïdienne du nouveau-né. De même, au Kivu, où l'apport iodé des femmes enceintes est le même qu'à Bruxelles mais où l'apport en SCN est plus élevé, les taux de TSH au cordon sont très significativement augmentés et ceux de  $T_4$  abaissés.

Dans les conditions physiologiques comme à Kinshasa, il n'existe pas de corrélations entre les taux de  $T_4$ ,  $T_3$  et TSH mesurés chez la mère et le nouveau-né. Par contre, de telles corrélations sont observées dans les zones rurales du Zaïre et surtout en Ubangi (Tableau 12). Elles témoignent probablement de l'action des mêmes facteurs alimentaires d'environnement chez la mère et chez le fœtus.

En conclusion, ce travail montre que la femme enceinte et surtout le nouveau-né sont plus sensibles à l'action antithyroïdienne du manioc que la population générale vivant dans les mêmes conditions d'environnement. Le nouveau-né constitue la fraction cible de cette population. Par conséquent, le dépistage de l'hypothyroïdie congénitale constitue la méthode la plus sensible en vue de détecter le risque d'altération de la fonction thyroïdienne et de retard mental résultant de la consommation de manioc dans une population donnée.



In humans, the transfer of thyroid hormones and of TSH across the placenta is practically negligible (Fisher et al. 1964, 1977b; Dussault et al. 1969; Burrow 1972). Therefore, the serum concentrations of these hormones in cord blood depend solely on the function of the fetal thyroid and on the thyroid-hypothalamus-pituitary feedback mechanism of the fetus (Fisher and Dussault 1974; Fisher et al. 1977b; Fisher 1980). Consequently, under physiological conditions, the concentrations of serum thyroid hormones and TSH in pregnant women at delivery are unrelated to those in cord blood (Yamazaki et al. 1961; Denayer et al. 1966; Robin et al. 1970; Czernichow et al. 1971; Dokumov et al. 1971; Montalvo et al. 1973; Burman et al. 1976; Fisher et al. 1977b; Delange et al. 1980c; Dussault et al. 1980; Fisher and Klein 1981).

Iodide, however, crosses the placenta (Logothetopoulos and Scott 1955; Hall and Myant 1956; Lybeck and Hirvonen 1956; Brown-Grant 1961; French and Van Wyck 1964; Book et al. 1974; Iancu et al. 1974; Job et al. 1974) and the iodine stores of the fetal thyroid depend critically on the iodide supply from the mother (Palmer et al. 1938; Malvaux et al. 1969; Delange et al. in press).

There are few studies on thyroid function of the newborn in endemic goitre areas with severe iodine deficiency. Pilot studies in Peru (Pretell et al. 1974), Algeria (Bachtarzi 1979), and Sicily (Vigneri et al. 1980) have shown that, as compared to control areas with adequate iodine supply, newborns had slightly elevated TSH and low  $T_4$ . However, none of these studies showed the biochemical profile of severe thyroid failure observed in sporadic congenital hypothyroidism.

In contrast, data from the Ubangi endemic goitre area indicated that such a profile was observed in about 10% of the newborns (Delange et al. 1976, 1980a; Thilly et al. 1978); yet the degree of iodine deficiency in this area was practically the same as that observed in the pilot studies mentioned.

The additional role of cassava was suspected because, in Ubangi, both mothers and newborns had markedly elevated levels of serum SCN (Ermans et al. 1980a). Moreover, the previously reported (Delange et al. 1980a) correlation between the SCN concentrations in pregnant mothers and in cord blood indicated that SCN crosses the placenta.

Therefore, because the role played by cassava in the induction of thyroid failure in the newborn in the Ubangi area had not been

clearly demonstrated, the present study was designed:

- To clarify the influence of the balance between the dietary supplies of iodine and SCN in pregnant women on the thyroid function of the newborn.
- To assess the role played by SCN of maternal origin on this function.
- To compare the relationship between thyroid function in pregnant women at delivery and that in the newborn in normal conditions and in severe endemic goitre.

The study consisted of a comparison of the biochemical characteristics of thyroid function and of SCN metabolism in pregnant women and newborns in Brussels and in four different areas in Zaire with strikingly different dietary supplies of iodine and cassava. The serum concentrations of total proteins and albumin were also determined to evaluate the possible role of the dietary supply of proteins.

## Patients and Methods

The study was carried out in the maternity clinics of Hôpital Saint-Pierre in Brussels, Clinique Ngaliema and Dispensaire de Bumbu in Kinshasa, Hôpital de Kisantu in Bas Zaire, Hôpital de Kabare in Kivu, and Hôpital de Karawa in Ubangi. In Kinshasa, the Clinique Ngaliema was attended by the upper socioeconomic class of Kinshasa while the Dispensaire Bumbu, located in the suburbs of the city, was attended by the lower socioeconomic class. The maternity clinics in Bas Zaire, Kivu, and Ubangi were attended by representative samples of the total population. However, some mothers in Ubangi had urinary iodine concentrations that were significantly higher than in the general population of the same region (see Tables 7 and 9). This situation probably resulted from more frequent medical care in some pregnant women attending the prenatal clinics, including "silent" iodine prophylaxis. For this reason, the pregnant women in the Ubangi area were divided into two groups — the total population and those with a urinary iodine level lower than  $4 \mu\text{g}/\text{dl}$ , i.e., with a value within the range for the general population of the Ubangi area and indicating severe iodine deficiency.

The study included 637 mothers investigated at delivery and 674 newborns. The distribution of the patients in the seven different groups is indicated in Table 9 and Fig. 8.

Blood and casual urine samples in the mothers and cord blood samples were collected at delivery for the determination of concentrations of serum  $T_3$ ,  $T_4$ , TSH, SCN, total proteins, and albumin, and of urinary creatinine, iodine, and SCN, and for calculation of urinary I/SCN ratios.

## Results

Table 9 compares the results obtained in the mothers for thyroid function and for iodine and SCN supplies. Compared to the upper socioeconomic class of Kinshasa, the mothers in Brussels had significantly higher serum and urinary concentrations of SCN and lower urinary concentrations of iodine, and consequently much lower urinary I/SCN ratios. Serum TSH and  $T_4$  were similar in the two groups but  $T_3$  was slightly lower in Brussels. Since these results indicated that the iodine and SCN dietary supplies in the upper class of Kinshasa were more adequate than in Brussels, the results for the different groups in Zaire were compared to those for the Clinique Ngaliema in Kinshasa.

In passing from Kinshasa through Bas Zaire and Kivu to Ubangi, there was a progressive increase in the concentrations of serum and urinary SCN and a decrease in the concentrations of urinary iodine, and consequently in the urinary I/SCN ratios. Compared to the control group, serum SCN was doubled in the lower social class of Kinshasa (Bumbu) and in Bas Zaire and Kivu, and increased by a factor of four in Ubangi. The urinary I/SCN ratios were slightly, but not significantly, lower in Bumbu and Bas Zaire than in Kinshasa. In Kivu and Ubangi, they were 3.5 and 3.3 times lower than in the control group respectively. We must point out that the concentrations of urinary iodine observed in Brussels were not significantly different from those observed in Bas Zaire and Kivu and were only slightly higher than in Ubangi ( $P < 0.05$ ).

In parallel to the progressive decrease of the urinary I/SCN ratios, concentrations of serum TSH increased progressively and those of serum  $T_4$  decreased. TSH was significantly higher than in the control group from Bas Zaire onwards and was 4.7 times higher in Ubangi.  $T_3$  was barely changed. In the Ubangi area, the severely iodine-deficient group had lower  $T_4$  and higher  $T_3$  values than in the total population of pregnant women. TSH, however, reached the same high concentrations.

Table 10 shows the concentrations of serum total proteins and albumin in the mothers and

newborns, the urinary concentrations of creatinine in the mothers, and the weights at birth of the newborns. The concentrations of serum albumin in cord blood were practically identical in the seven groups and were consistently slightly higher than in the mothers. For the mothers, they were also very similar in all groups except in the low socioeconomic class in Kinshasa and in Ubangi where they were lower than in the control group. In the three rural regions, the concentrations of serum total proteins, both in mothers and newborns, were similar or significantly higher than in the control group. Moreover, they were systematically higher in all the groups of pregnant women in Zaire than in Brussels ( $P < 0.01$ – $0.001$ ). The weights at birth were lower than in Kinshasa in the other Zairian groups with the lowest value being observed in Ubangi. The values for Brussels and the upper class of Kinshasa were similar.

The results obtained for SCN,  $T_3$ ,  $T_4$ , and TSH in cord blood, are shown in Fig. 8. As in the mothers, SCN level increased progressively and significantly from Kinshasa to Ubangi. In parallel, TSH increased and  $T_4$  decreased. However, contrasting with the situation observed in the mothers, TSH levels in newborns of the Ubangi area were nine times higher than in the control group and  $T_3$  levels were doubled. As in the mothers, newborns of the Ubangi area born to severely iodine-deficient mothers had lower  $T_4$  and higher  $T_3$  than in the total newborn population of the Ubangi area.

Fig. 9 shows the distribution of the individual values obtained for TSH and  $T_4$  in cord blood in Brussels, Kinshasa (upper socioeconomic class), Bas Zaire, Kivu, and Ubangi (total newborn population). The limits indicated by the dotted lines in this figure for TSH and  $T_4$  were selected on the following basis:  $20 \mu\text{U}/\text{ml}$  for TSH and  $7 \mu\text{g}/\text{dl}$  for  $T_4$  correspond to the mean values observed in Brussels in cord blood ( $8.2 \mu\text{U}/\text{ml}$  for TSH and  $11.4 \mu\text{g}/\text{dl}$  for  $T_4$ ) plus or minus 2 SD;  $50 \mu\text{U}/\text{ml}$  for TSH and  $5 \mu\text{g}/\text{dl}$  for  $T_4$  are used as cut-off points in the screening programs for congenital hypothyroidism in cord blood in Western countries. The values of  $100 \mu\text{U}/\text{ml}$  or more for TSH and  $3 \mu\text{g}/\text{dl}$  or less for  $T_4$  are usually considered as indicative of the presence of sporadic congenital hypothyroidism. The percentages of newborns in each group with a cord TSH value lower than  $20 \mu\text{U}/\text{ml}$  or a cord  $T_4$  value higher than  $7 \mu\text{g}/\text{dl}$  are shown in the same figure.

The percentages of infants with TSH and  $T_4$  in cord blood within these limits showed a pro-

Table 9. Comparison of concentrations of serum SCN, thyroid hormones, and TSH, of urinary iodine and SCN, and of the urinary I/SCN ratios in pregnant women at delivery in Brussels, Kinshasa (upper and lower socioeconomic groups), Bas Zaire, Kivu, and Ubangi (total population of women at delivery and severely iodine-deficient women). (Mean  $\pm$  SEM.<sup>a</sup> Numbers of patients are shown in parentheses.)

Variables	Brussels	Kinshasa		Bas Zaire	Kivu	Ubangi	
		Upper group	Lower group			Total	Severely I deficient
Serum concentration							
SCN (mg/dl)	0.35±0.03*** (111)	0.19±0.01 (98)	0.38±0.03*** (22)	0.40±0.03*** (53)	0.38±0.02*** (144)	0.76±0.03*** (170)	0.78±0.05*** (98)
T <sub>4</sub> (μg/dl)	12.7±0.3NS (112)	12.5±0.2 (99)	10.6±0.4*** (22)	11.7±0.3* (53)	11.3±0.2*** (146)	8.6±0.4*** (193)	8.0±0.5*** (117)
T <sub>3</sub> (ng/dl)	182±5*** (109)	207±6 (97)	190±11NS (21)	198±7NS (52)	219±4NS (145)	189±5* (197)	196±6NS (118)
TSH (μU/ml)	3.6±0.2NS (111)	3.4±0.2 (91)	3.9±0.4NS (22)	4.3±0.3** (53)	4.1±0.2** (145)	15.8±2.4*** (197)	14.0±2.4*** (120)
Urinary concentration							
I (μg/dl)	4.4±0.3** (119)	6.1±0.5 (64)	8.2±1.2NS (14)	4.6±0.4* (41)	3.9±0.2*** (140)	3.5±0.2*** (181)	1.8±0.09*** (130)
SCN (mg/dl)	1.03±0.05* (120)	0.84±0.04 (99)	1.14±0.15NS (21)	0.89±0.09NS (51)	1.14±0.05** (146)	1.52±0.09*** (193)	1.41±0.09*** (128)
Urinary ratio							
I/SCN (μg/mg)	5.0±0.4** (119)	12.1±2.8 (64)	10.0±2.4NS (13)	7.0±0.5NS (41)	3.5±0.1*** (139)	3.7±0.5** (176)	2.3±0.2*** (128)

<sup>a</sup>Levels of significance refer to the comparison with the results from the upper socioeconomic group in Kinshasa: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

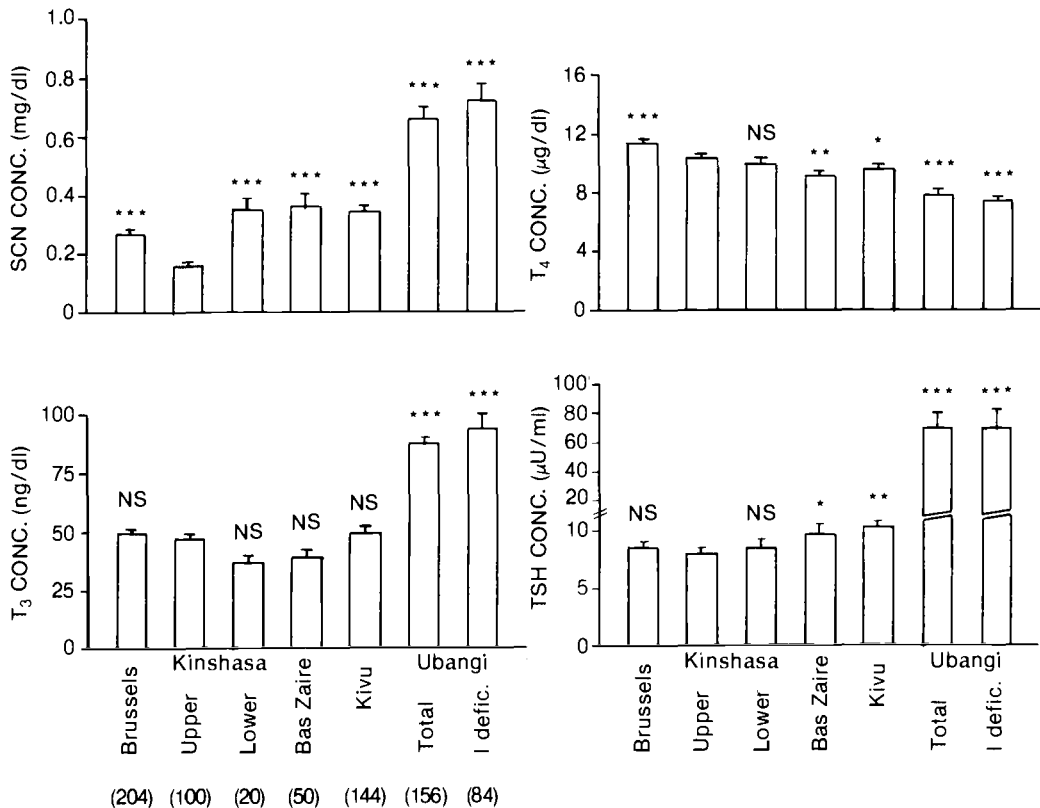
Table 10. Comparison of concentrations of serum total proteins and albumin in mothers at delivery and in newborns, of concentrations of urinary creatinine in the mothers and of the weight at birth in Brussels and in the same areas in Zaire as in Table 9.  
(Mean  $\pm$  SEM. <sup>a</sup> Numbers of patients are shown in parentheses.)

Variables	Brussels	Kinshasa		Bas Zaire	Kivu	Ubangi	
		Upper group	Lower group			Total	Severely I deficient
<b>Mother</b>							
Serum concentration							
Proteins (g/dl)	5.97±0.07*** (110)	6.46±0.08 (98)	6.46±0.19NS (22)	7.05±0.11*** (52)	6.47±0.06NS (141)	6.78±0.11* (120)	6.55±0.16NS (66)
Albumin (g/dl)	3.38±0.05NS (110)	3.47±0.05 (98)	3.20±0.09** (22)	3.30±0.07* (52)	3.38±0.04NS (141)	3.20±0.05*** (120)	3.12±0.07** (67)
Urinary concentration							
Creatinine (mg/dl)	-	113±18 (22)	-	102±12NS (29)	128±15NS (28)	-	-
<b>Newborn</b>							
Birth weight (kg)	3.20±0.01NS (7220) <sup>b</sup>	3.21±0.07 (57)	2.91±0.07** (21)	2.82±0.07*** (52)	2.94±0.05** (96)	2.69±0.02*** (160) <sup>c</sup>	2.63±0.05*** (112) <sup>c</sup>
Serum concentration							
Proteins (g/dl)	5.70±0.07NS (192)	5.75±0.08 (94)	5.98±0.21NS (20)	5.78±0.12NS (49)	6.09±0.06*** (144)	6.20±0.09*** (119)	6.03±0.12*** (64)
Albumin (g/dl)	3.73±0.04NS (192)	3.81±0.05 (94)	3.84±0.13NS (20)	3.69±0.07NS (49)	3.88±0.04NS (144)	3.79±0.06NS (119)	3.68±0.08NS (64)

<sup>a</sup>Levels of significance refer to the comparison with the results from the upper socioeconomic group in Kinshasa: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

<sup>b</sup>From Delange et al. (1979).

<sup>c</sup>From Thilly (1979).



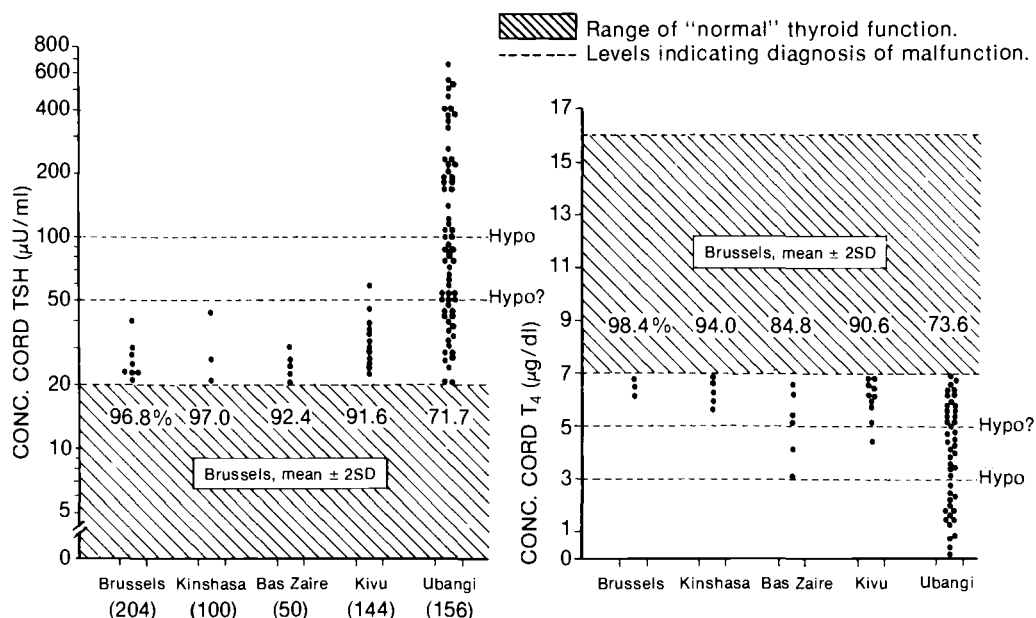
**Fig. 8.** Comparison of concentrations of serum SCN, T<sub>4</sub>, T<sub>3</sub>, and TSH in cord blood in Brussels and in the same areas in Zaire as in Table 9. (Mean  $\pm$  SEM. Numbers of patients are shown in parentheses. Levels of significance refer to the comparison with the results from upper socioeconomic group in Kinshasa: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .)

gressive decrease from Brussels and Kinshasa to Ubangi, where 28.3% were beyond the limit for TSH and 26.4% for T<sub>4</sub>. The clearcut trend of these percentages in Bas Zaire and Kivu are of particular interest considering the slight or very slight modifications of the mean concentrations of TSH and T<sub>4</sub> observed in these regions in cord blood (Fig. 8) and in the mothers (Table 9). Dramatic abnormalities were observed in the newborns in Ubangi: 19.0% of them had a cord TSH value higher than 50  $\mu$ U/ml and 12.1% had values equal to or higher than 100  $\mu$ U/ml, ranging up to 628  $\mu$ U/ml; 15.1% had cord T<sub>4</sub> values lower than 5  $\mu$ g/dl and 13.8% of them had values lower than 3  $\mu$ g/dl; 11.2% of the newborns had both a cord TSH higher than 100  $\mu$ U/ml and a cord T<sub>4</sub> lower than 3  $\mu$ g/dl.

The serum concentrations of TSH and T<sub>4</sub> in cord blood are compared in Table 11 for two subgroups of the severely iodine-deficient group from the Ubangi area where values of

both urinary iodine and SCN were available for the mothers at delivery. The two subgroups were formed on the basis of the concentrations of urinary SCN in the mothers, using a cut-off point of 1.2 mg/dl, which corresponded to the mean value (0.84 mg/dl) plus 1 SD in the control group of Kinshasa. The iodide concentrations in the mothers were not significantly different while, as a consequence of the selection of the subgroups, urinary SCN was markedly higher in subgroup 2 than in subgroup 1. Mean cord T<sub>4</sub> and TSH were lower and substantially higher, respectively, in subgroup 2 than in subgroup 1, but the difference was only significant for T<sub>4</sub>. However, considering a cut-off of 5  $\mu$ g/dl for T<sub>4</sub> and of 50  $\mu$ U/ml for TSH, the two subgroups differed for both T<sub>4</sub> and TSH ( $\chi^2 = 6.567$  and 3.861, respectively: df 1,  $P < 0.05$ ).

Table 12 shows the coefficients of correlation obtained in each region between the serum concentrations of T<sub>3</sub>, T<sub>4</sub>, and TSH in the moth-



**Fig. 9.** Distributions of serum TSH and  $T_4$  values in cord blood in Brussels, Kinshasa (upper socioeconomic group), Bas Zaire, Kivu, and Ubangi (total group). Percentages indicate proportion of newborn population that falls within "normal" range (mean for Brussels  $\pm$  2 SD). (Numbers of newborns are shown in parentheses.)

Table 11. Comparison of concentrations of serum TSH and  $T_4$  in cord blood in newborns born to severely iodine-deficient mothers with slight (group 1) or marked (group 2) elevation of urinary SCN concentrations at delivery. (Mean  $\pm$  SEM. <sup>a</sup> Numbers of patients are shown in parentheses.)

Variables	Group 1	Group 2
Mothers — Urinary concentration		
I ( $\mu\text{g}/\text{dl}$ )	2.0 $\pm$ 0.1 (49)	2.2 $\pm$ 0.1NS (60)
SCN (mg/dl)	0.70 $\pm$ 0.05 (48)	2.12 $\pm$ 0.01*** (60)
Newborns — Serum concentration		
TSH ( $\mu\text{U}/\text{ml}$ )	48.0 $\pm$ 15.5 (36)	95.5 $\pm$ 25.2NS (34)
$T_4$ ( $\mu\text{g}/\text{dl}$ )	8.3 $\pm$ 0.6 (37)	6.3 $\pm$ 0.6* (34)

<sup>a</sup>Levels of significance: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

ers and in the newborns. These correlations explored separately the thyroid-pituitary feedback mechanisms of the mothers and the newborns. Both in mothers and newborns, there were significant direct correlations between the concentrations of serum  $T_4$  and  $T_3$  except in the Ubangi area where there was no

correlation in the mothers and an indirect correlation in the newborns. There were no significant correlations, either in mothers or newborns between TSH and  $T_3$ , except in the control group, or between TSH and  $T_4$ , except in the Ubangi area where there was a highly significant indirect correlation.

Table 13 and Fig. 10 show the coefficients of correlation found between the variables measured in the mothers and their newborns. These correlations explored the fetomaternal relationships. Fig. 10 shows that, for the total population from the seven groups, there was an extremely significant direct correlation between the serum concentrations of SCN in the mother and in cord blood ( $r = 0.908$ ,  $P < 0.00001$ ). Table 13 shows that there were also highly significant direct correlations between  $T_3$ ,  $T_4$ , and TSH in the mother and the newborn in the Ubangi area, as well as an indirect correlation between  $T_4$  in the mother and  $T_3$  and TSH in the newborn. In the control group, there were no correlations between the variables measured in the mothers and in the newborns except for  $T_3$  and  $T_4$ .

We must point out that, in the Brussels group, there were also correlations between  $T_4$

Table 12. Coefficients of correlation<sup>a</sup> between the serum concentrations of T<sub>3</sub>, T<sub>4</sub>, and TSH in the mothers at delivery and in the newborns.

Variables	Mothers		Newborns	
	T <sub>3</sub>	T <sub>4</sub>	T <sub>3</sub>	T <sub>4</sub>
T <sub>4</sub>				
Kinshasa	0.306**		0.570***	
Brussels	0.258**		0.379***	
Bas Zaire	0.416**		0.756***	
Kivu	0.372**		0.288***	
Ubangi	NS		-0.239***	
TSH				
Kinshasa	0.272**	NS	0.317***	NS
Brussels	NS	NS	NS	NS
Bas Zaire	NS	NS	NS	NS
Kivu	NS	NS	NS	NS
Ubangi	NS	-0.381***	NS	-0.622***

<sup>a</sup>Levels of significance: NS, nonsignificant; \*\*, P < 0.01; \*\*\*, P < 0.001.

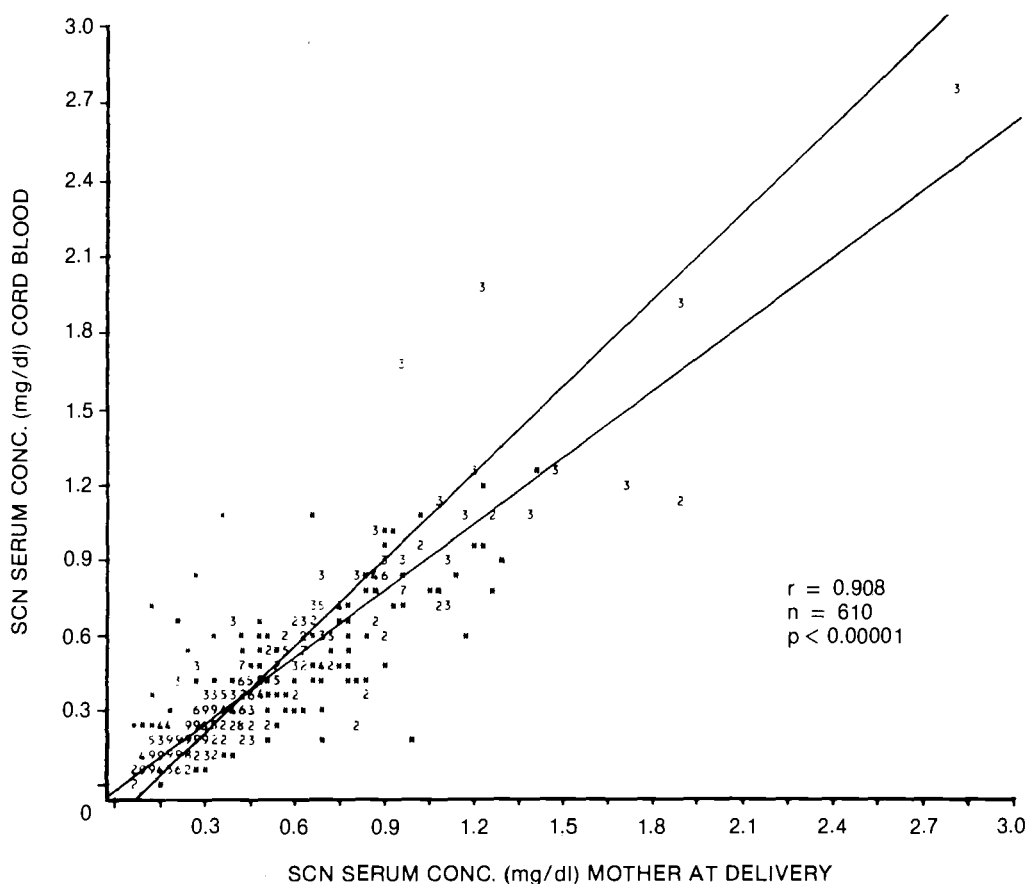


Fig. 10. Relationship between concentrations of serum SCN in the mother at delivery and in cord blood of infants for the seven groups investigated. (Numbers of patients: \* = 1, 2-8 = 2-8, 9 = ≥9.)

Table 13. Coefficients of correlations<sup>a</sup> between concentrations of T<sub>3</sub>, T<sub>4</sub>, and TSH and urinary I and SCN in the mothers at delivery and the same serum variables in the newborns.

Newborns	Mothers				
	Serum concentration			Urinary concentration	
	T <sub>3</sub>	T <sub>4</sub>	TSH	I	SCN
T <sub>3</sub>					
Kinshasa	0.272**	NS	NS	NS	NS
Brussels	NS	NS	NS	NS	NS
Bas Zaire	NS	NS	NS	NS	NS
Kivu	NS	NS	NS	NS	NS
Ubangi	0.351***	-0.403***	NS	-0.237**	NS
T <sub>4</sub>					
Kinshasa	NS	0.216*	NS	0.322**	NS
Brussels	NS	0.340***	NS	NS	NS
Bas Zaire	NS	0.292*	NS	NS	NS
Kivu	NS	0.188*	NS	NS	NS
Ubangi	NS	0.603***	-0.342***	0.229*	-0.271**
TSH					
Kinshasa	NS	NS	NS	NS	NS
Brussels	0.302**	NS	0.362***	NS	NS
Bas Zaire	NS	NS	0.292*	NS	NS
Kivu	NS	0.240**	NS	NS	NS
Ubangi	NS	-0.561***	0.438***	NS	NS

<sup>a</sup>Levels of significance: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

in the mother and T<sub>4</sub> in the newborn and between TSH in the mother and TSH in the newborn.

Concentrations of urinary iodine and SCN in the mothers and the same variables measured in cord blood were not correlated except in Ubangi where T<sub>4</sub> in cord blood was correlated to the maternal concentration of urinary iodine (directly) and of urinary SCN (indirectly). There were no correlations between the concentrations of serum SCN either in the mothers or in the newborns with T<sub>4</sub>, T<sub>3</sub>, and TSH in cord blood.

## Discussion

The results demonstrate that the thyroid function of the newborn is critically influenced by modifications of the iodine and SCN supplies of the mother. They also demonstrate that newborns are much more sensitive to the goitrogenic action of iodine deficiency and SCN than is the general adult population.

Pregnant women in the upper socioeconomic group in Kinshasa have a urinary I/SCN ratio of 12.1, a value far above the critical level of 3–4 resulting in the development of endemic

goitre. The low socioeconomic class of Kinshasa has concentrations of serum SCN twice as high as the upper class, probably resulting from a higher dietary intake of cassava. Because of their high iodine intake, the mothers of this group still have a high urinary I/SCN ratio and the concentrations of serum TSH and T<sub>4</sub> in the newborns remain comparable to those observed in the control group.

In Bas Zaire, the serum SCN concentrations in cord blood are identical to those observed in the lower class of Kinshasa, but the iodine intake of the mothers is slightly lower. As a result, newborns in Bas Zaire have higher TSH and lower T<sub>4</sub> values than those in the control group. These modifications occur with a I/SCN ratio of 7, i.e., still much higher than the critical threshold reported for adult population and higher than the value of 3.7 reported for the adult population of the same area, which has no abnormal prevalence of goitre.

In the Kivu area, with the same SCN concentrations at birth as in Bas Zaire but with a still lower iodine intake, the modifications of TSH and of T<sub>4</sub> in mothers and newborns are more important than in Bas Zaire in spite of a borderline I/SCN ratio (3.5), which is higher than in the adult population of the area (3.0) in whom serum TSH is not modified.



In the Ubangi area, the serum SCN in the newborn is four times higher than that in the controls and the maternal urinary iodine concentration is nearly half, with extreme modifications of cord TSH and  $T_4$ . Once more, the I/SCN ratio in pregnant mothers (3.7) is more than twice the ratio in the general adult population of the same area (1.6).

Thus, in Bas Zaire, Kivu, and Ubangi, there is a progressive alteration of thyroid function of the newborn that is much more pronounced than in the adult populations of the same areas in spite of the fact that the mothers have urinary I/SCN ratios higher than in the general population. This last observation results mainly from the fact that pregnant women had lower concentrations of urinary SCN than the general adult populations, while their iodine concentration was almost identical, except in Ubangi, as indicated by a comparison of Tables 7 and 9.

The role played by SCN overload in the impairment of thyroid function of the newborn is clearly shown in Ubangi where higher maternal urinary SCN concentrations than in Kivu, but identical urinary iodine concentrations (Table 9), are accompanied by extreme modifications of cord TSH and  $T_4$  (Fig. 9). Also, the highest urinary SCN values in severely iodine-deficient mothers in Ubangi are accompanied by a further increase of TSH and decrease of  $T_4$ . Also in agreement with this view is the observation that, in Kivu, higher urinary SCN in mothers than in Brussels, with a non-significant difference in urinary iodine concentration (Table 9), is accompanied by significantly higher cord TSH and lower cord  $T_4$  (Figs. 8 and 9).

We must note that the higher serum SCN concentrations in pregnant women in Brussels — twice the level in the upper class of Kinshasa — probably result from smoking (Andrews 1973; Butts et al. 1974; Pettigrew et al. 1977) during pregnancy. They also have a lower iodine supply and, consequently, their I/SCN ratios are half the Kinshasa controls ( $P < 0.01$ ). The possibility that Belgian newborns are at risk of thyroid failure is suggested by the occurrence of transient primary hypothyroidism in 1 in 700 newborns in Brussels (Delange et al. 1979).

The hypersensitivity of the newborn to the antithyroid action of SCN probably results from the fact that SCN interferes with the trapping of iodide by the placenta (Logothetopoulos and Scott 1955) and by the newborn's own thyroid gland (Wolff 1964). These two factors

might indeed reduce the iodine supply from the mother to the thyroid of the fetus and critically reduce the buildup of iodine stores within the gland, which takes place mainly during the fetal and early postnatal life (Palmer et al. 1938; Malvaux et al. 1969; Stolc et al. 1973; Delange et al. in press). It has been shown that the concentration of iodine per unit of weight of the thyroid gland plays a determining role in the efficiency of hormonal synthesis and secretion by the thyroid (Ermans et al. 1963, 1968; Ermans 1969). Reduction of this concentration results in an increased  $T_3/T_4$  ratio within the gland. This modification probably accounts for the markedly increased serum  $T_3$  concentrations found in newborns in Ubangi compared to those of the controls and indicates that, in severe endemic goitre, the newborn is, as are the child and the adult (Delange et al. 1972a; Kochupillai et al. 1973; Patel et al. 1973; Pharoah et al. 1973; Vagenakis et al. 1973; Chopra et al. 1975; Suwanik et al. 1975), able to compensate, at least partly, for the effect of iodine deficiency by increasing the release of  $T_3$  from the thyroid. This compensation also probably explains the lack of correlation between TSH and  $T_3$  in cord blood, and precludes the hypothesis of preferential secretion of  $T_3$  under TSH hyperstimulation. The indirect correlation found between cord  $T_4$  and  $T_3$  also precludes the possibility that increased  $T_3$  concentrations result from increased peripheral monodeiodination of  $T_4$  to  $T_3$  (Gavin et al. 1977).

In Western countries, a cord TSH value higher than  $100 \mu\text{U/ml}$  associated with a cord  $T_4$  value lower than  $3 \mu\text{g/dl}$  is considered as typical for sporadic congenital hypothyroidism (Dussault et al. 1980; Delange et al. 1980c). According to these criteria, this study confirms that the incidence of congenital hypothyroidism in the newborn in the Ubangi area is 11% (Thilly et al. 1978; Delange et al. 1980a) — 400 times higher than in Western countries. This situation probably plays a determining role in the etiology of endemic mental retardation and may be the most important public health problem of the affected population. However, we cannot exclude the possibility that newborns are partly protected against the effects of hypothyroidism on brain development by drastically increased concentrations of serum  $T_3$ . This mechanism could partly explain the observation that the incidence of congenital hypothyroidism detected at birth is markedly higher than the prevalence of myxedematous endemic cretinism determined in the general

population (Lagasse et al. 1980a; see also chapter 2).

Our work confirms that, in physiological conditions such as those observed in the upper socioeconomic group of Kinshasa, there are no correlations between  $T_3$ ,  $T_4$ , and TSH measured in the mother at delivery and in cord blood (Fisher et al. 1977b). It also confirms that, in contrast, such correlations are found in the presence of important goitrogenic factors in the diet (Thilly et al. 1978). These findings are probably related to the action of the same environmental factors on both the mother and the newborn. They also suggest that, in these particular conditions, the determination of TSH and  $T_4$  in pregnant women may constitute an index of the risk of thyroid failure in the newborn (Thilly et al. 1978).

It is interesting to observe that, in Brussels, where the I/SCN ratio in pregnant women is borderline, there are also correlations between  $T_4$  in the mother and  $T_4$  in the newborn and between TSH in the mother and TSH in the newborn. These observations further stress the borderline situation of the Belgian newborns regarding thyroid function (Delange et al. 1980d).

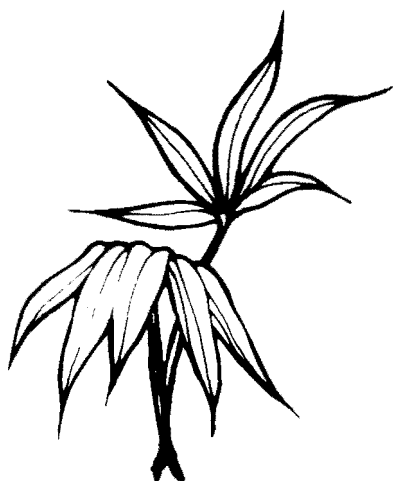
Despite extremely marked differences in the prevalence of clinical and biochemical signs of malnutrition in the general populations investigated, albumin concentration in cord blood is remarkably uniform. Thus, this variable is a poor index of protein malnutrition for a given population. However, the influence of a less adequate nutrition status of pregnant women could be reflected by lower birth weights ob-

served in the different groups compared to Kinshasa. The lowest value, observed in Ubangi, could also result from the direct effect of fetal hypothyroidism, as previously suggested by Thilly (1979).

The levels of total proteins vary drastically from one area to another, possibly reflecting differences in the prevalence of infections. Our results also confirm that the concentrations of serum total proteins and albumin in pregnant women and in cord blood are lower than in nonpregnant women (Mack 1955).

In conclusion, this study shows that pregnant women and, especially, newborns are more sensitive to the antithyroid action of goitrogens of dietary origin than children and adults living under the same nutritional conditions. In populations where the I/SCN ratio is only slightly lowered and where thyroid function in the general population is unaffected, there is a definite shift of the TSH and  $T_4$  levels in cord blood towards high and low values, respectively. When the I/SCN ratio in pregnant women reaches 3.7, a figure corresponding only to the threshold value for the development of goitre in a general population, the changes are dramatic and about 10% of the newborns exhibit a caricatural biochemical picture of congenital hypothyroidism.

This investigation thus shows that pregnant mothers and newborns are particularly vulnerable to the action of cassava on thyroid function. Consequently, systematic screening for congenital hypothyroidism constitutes the most sensitive index for detecting the risk of mental retardation resulting from cassava consumption in iodine-deficient areas.



## Chapter 5

# Cassava Products: HCN Content and Detoxification Processes

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**Dérivés du manioc : contenu en cyanure et processus de détoxification — Résumé —** Il existe dans les populations du Bas-Zaïre, du Kivu et de l'Ubangi une surcharge en SCN qui résulte de la consommation chronique de manioc. L'importance de cette surcharge et les modes de préparation du manioc sont très différents dans ces trois régions. Une étude comparative a donc été effectuée avec comme objectifs : 1) d'apprécier le contenu en HCN du manioc cru et des aliments dérivés du manioc; 2) d'analyser en laboratoire l'influence des processus de détoxification du manioc sur son contenu en HCN; et 3) de tenter de diminuer la surcharge en HCN chez des habitants de l'Ubangi en modifiant leurs habitudes alimentaires.

L'analyse du contenu en HCN des tubercules (produit par l'hydrolyse de la linamarine) prove-

nant des trois régions montre une dispersion très importante dans chaque région — 2–309 mg HCN/kg en Ubangi (180 échantillons), 12–205 mg HCN/kg au Kivu (28 échantillons) et 5–142 mg HCN/kg dans le Bas-Zaïre (25 échantillons). Il n'existe pas de corrélation évidente entre le contenu en HCN et les critères morphologiques. Cependant, il apparaît que le pourcentage de tubercules considérés comme toxiques

(> 100 mg HCN/kg) est faible (4 %) dans le Bas-Zaïre mais plus élevé et sensiblement identique (21 et 24 %) au Kivu et en Ubangi (Tableau 14).

D'autre part, l'analyse du contenu en HCN des aliments consommés par les populations des trois régions laisse apparaître des taux de HCN très faibles dans le Bas-Zaïre (fufu : < 1,0 et chickwan-gue : 1,3 mg HCN/kg), intermédiaires au Kivu (bugali : 6,3 mg HCN/kg) et relativement élevés en Ubangi (fuku : 17,3 et mpondu : 8,2 mg HCN/kg) (Tableau 16).

L'étude des processus de détoxification utilisés par les populations de ces trois régions montre que les variations des taux de HCN sont étroitement liées à des méthodes de préparation alimentaires différentes. Ainsi, le séchage au soleil, méthode de détoxification quasi générale en Ubangi, ne produit en réalité qu'une déshydratation partielle des tubercules avec une élimination fort incomplète ( $\pm 80$  %) du HCN initialement présent dans le manioc (Tableau 19). La combinaison du séchage au soleil et d'une étape de fermentation, fort utilisée au Kivu, produit des aliments dont le contenu en HCN bien que non négligeable, est nettement plus faible que celui observé en Ubangi. Le rouissage du manioc en une ou deux étapes, largement pratiqué dans le Bas-Zaïre, constitue vraisemblablement le procédé de détoxification le plus efficace, la teneur en HCN des aliments consommés dans cette région étant de loin la plus faible (Tableau 17).

Une étude réalisée en laboratoire sur des tubercules amers collectés dans l'Ubangi, confirme que l'utilisation de techniques de détoxification plus poussées (par exemple le rouissage) permet l'obtention d'aliments dont le taux en HCN est négligeable (Tableau 18).

Dans l'Ubangi, une tentative pour diminuer l'apport en HCN en modifiant les habitudes alimentaires d'une famille très motivée a échoué (Fig. 11). Ceci confirme l'extrême difficulté d'introduire de telles modifications au sein des populations rurales.

En conclusion, les différences observées dans les trois régions rurales investiguées au Zaïre concernant le contenu en HCN des aliments dérivés du manioc proviennent en partie de différences dans le contenu en HCN du manioc cru mais surtout de

différences dans les processus de détoxification utilisés. Le rouissage apparaît comme la méthode la plus efficace. Une augmentation de la consommation de manioc et/ou une diminution de l'efficacité des processus de détoxification est susceptible d'entraîner l'apparition de troubles de la fonction thyroïdienne dans des régions actuellement non affectées.

The studies reported in chapters 2, 3, and 4 show that the rural populations of Bas Zaire, Kivu, and Ubangi have markedly higher concentrations of serum and urinary SCN than do control populations in Kinshasa and Brussels. The overload resulted from chronic intake of cassava products. These studies also show important differences among the concentrations of serum and urinary SCN from one region of Zaire to another as well as in the methods of preparing cassava-based meals.

The question therefore arose of whether these variations in SCN overload were caused by differences in the HCN content of fresh cassava or in the methods used to prepare the cassava products, or both, (De Bruijn 1971; Simons-Gérard et al. 1980).

A comparative study was carried out, therefore, in the three regions of Zaire to estimate the HCN content, first, of fresh roots collected locally and, secondly, of cassava-based meals prepared by the local inhabitants. As a third step, the various processing methods were reproduced in the laboratory at Gemena to assess their effectiveness in the detoxification of cassava. Finally, we attempted to decrease the SCN overload in inhabitants of Ubangi through nutrition education.

## Material and Methods

Fresh cassava roots and leaves as well as the different cassava products commonly prepared and eaten in Ubangi, Kivu, and Bas Zaire were obtained from local inhabitants and analyzed for their HCN content. The preparation of the cassava products was studied in the three regions through house-to-house surveys and interviews.

In addition, cassava products were prepared in the laboratory at Gemena and the HCN content of the products determined at each step of the detoxification processes. A total of 739 samples were assayed for HCN determination. The HCN content reported for each sample in this study is the mean value obtained from five or six replicate assays of the same sample.

## Results

### HCN content of fresh cassava roots

The HCN content of fresh cassava roots collected in Ubangi ranged from 2 to 309 mg HCN/kg fresh weight (180 samples), in Kivu from 12 to 205 (28 samples), and in Bas Zaire from 5 to 142 (25 samples).

In all three regions, the wide range of individual results precluded calculating means. For this reason, and to allow more valid comparison between the results obtained in these regions, the individual values were classified into the three categories, based on HCN content, proposed by Bolhuis (1954), De Bruijn (1971), and Coursey (1979): *Innocuous*, less than 50 mg HCN/kg fresh peeled roots; *Moderately poisonous*, 50–100 mg HCN/kg; and *Dangerously poisonous*, over 100 mg HCN/kg.

The frequency distributions of HCN content of fresh roots among these three categories in Ubangi, Kivu, and Bas Zaire are shown in Table 14. The percentage of innocuous roots increased from 45% in Ubangi to 56 and 80% in Kivu and Bas Zaire, respectively. In contrast, the percentage of dangerously poisonous roots was almost identical in Ubangi (24%) and Kivu (21%) and was markedly lower in Bas Zaire (4%).

Table 14. Percentages of roots classified as innocuous (<50 mg HCN/kg fresh weight), moderately poisonous (50–100), and dangerously poisonous (>100) in the three areas.

Area	HCN content (mg/kg)		
	<50	50–100	>100
Bas Zaire	80	16	4
Kivu	56	22	21
Ubangi	45	31	24

As previously reported for Ubangi (Simons-Gérard et al. 1980), the measured HCN content of cassava roots did not correlate with the morphological criteria used by the inhabitants of the three regions to discriminate between sweet and bitter varieties. Consequently, no further attempt was made to distinguish between sweet and bitter varieties and the results obtained for the roots were pooled for each area.

We noted no morphological differences among roots from Bas Zaire and Ubangi but

roots and plants from Kivu were clearly different. The plants commonly cultivated in Kivu were smaller (30–50 cm high) and the vegetation less exuberant, the roots were also smaller (5–25 cm length) and more slender (3–5 cm diameter). In Bas Zaire, as in Ubangi, the roots were harvested after 6 months (sweet varieties) to 18 months (bitter varieties), whereas, in Kivu, young cassava stems were planted during September and the roots harvested in June to August of the next year for both sweet and bitter varieties.

As reported by many people living in Bas Zaire, the area was dramatically affected by drought during the previous 3 years. Moreover, many cassava plants were attacked by parasitoses locally known as "cochineal" or "cassava cholera."

### Preparation of cassava products

The methods of preparing food items containing cassava varied greatly among the three areas. The different cassava products commonly eaten in the three areas (Table 15) were prepared by six general methods. In addition, raw cassava roots (mainly sweet) were occasionally eaten by inhabitants of the three areas, mainly between meals.

**Boiled roots:** Fresh roots were peeled and boiled in water for 20–30 min until cooked.

**Maize and cassava gruel (fuku):** Fuku was only eaten in Ubangi where it was the basic foodstuff of the local population. As reported previously, bitter roots were peeled, cut into small pieces, and spread on the ground for 1–2 days to dry in the sun. Dried pieces were bruised in a wooden mortar with steeped (12–24 hours) maize and a flour was obtained. The amount

of maize added fluctuated with the period of the year. The flour was then gently heated on a pan and eaten as a gruel prepared with hot water.

**Cassava paste:** Fufu was the major constituent of diet in Bas Zaire. Bitter roots were soaked for 2–4 days (during the rainy season) or 4–6 days (during the dry season). They were peeled and soaked again for 1–2 additional days whenever possible, broken into small fragments, and finally sun-dried for 4–5 days. The grinding of the sun-dried pieces provided a flour that was boiled in water until a paste of firm and elastic consistency was obtained. Eventually, the paste was heated again in water and eaten like Italian polenta.

In Ubangi, the consumption of cassava paste, i.e., *fufu*, was only observed in urban areas. Small pieces of peeled roots, rarely soaked for 1–2 days, were sun-dried for 1–3 days and cooked in hot water until a consistent paste was obtained as in Bas Zaire.

In Kivu, cassava paste (*bugali*) was prepared from fresh roots that were peeled and sun-dried for 2–4 days. The roots were then buried in the earth for 4 days and sun-dried again for 2 additional days. Grinding and sieving gave a rather white flour that was boiled in hot water. When available, variable amounts of sorghum flour (obtained from ground grain) were added, giving the paste a brownish colour (*bugali ya mohogo na mutama*).

**Chickwangu:** As previously reported, *chickwangu* represented an important foodstuff only in the southern part of Ubangi. *Chickwangu* and *fufu* were rarely eaten by the villagers but were increasingly attractive foodstuffs in the urban communities. For example, in Gemena, the use of these products has in-

Table 15. Cassava products commonly eaten in Bas Zaire, Kivu, and Ubangi.

Cassava products	Local name		
	Bas Zaire	Kivu	Ubangi
Boiled roots	<i>Mateloko</i>	<i>Mohogo</i>	<i>Nsongo</i>
Maize and cassava gruel	—	—	<i>Fuku</i>
Cassava paste	<i>Fufu</i>	<i>Bugali ya mohogo</i>	<i>Fufu</i>
<i>Chickwangu</i>	<i>Nsua, Ntinga, and Nsesa</i>	—	<i>Kwanga</i>
Cassava leaves	<i>Nsaki, Kiselu</i>	<i>Sombe</i>	<i>Mpondu</i>
Sorghum and cassava paste	—	<i>Bugali ya mohogo na mutama</i>	—
Grilled roots	<i>Bikedi</i>	—	—

creased twofold during the past 5 years: the availability of regular salaries and the scarcity of cassava fields in an urban environment tended to increase the consumption of *chickwangué*, which could be bought ready to eat.

*Chickwangué* was prepared by soaking bitter roots for 2–6 days, and mashing them into a puree that was simmered to form a paste similar to *fufu*. The paste was wrapped up in a palm or banana leaf.

In Bas Zaire, *chickwangué* (*nsua*, *ntinga*, or *nseza*) was the most popular food after *fufu*. Its preparation required several days of work: bitter roots were soaked for 2–4 days (in the rainy season) or 4–6 days (dry season), peeled, soaked again for 1–2 days whenever possible, and ground after drying.

To prepare *nsua*, flour was mixed with water and filtered through a jute bag. After removal of the water, the paste was wrapped in a leaf and eaten raw.

*Ntinga* was prepared by mixing the flour with water and filtering it through a jute bag. The paste was then stored in the dark for 1–4 days. Half of the paste was then boiled in water and mixed with the remaining uncooked paste. The mixture was wrapped up in a leaf and cooked again.

To prepare *nseza*, cassava flour was mixed with water to form a paste. The lump of paste was covered with leaves and dried in the sun. After a few days, the leaves were removed and the paste was divided into "loaves" for subsequent sun-drying. The loaves were then ground to obtain a flour that was cooked like *fufu*, wrapped up in a leaf, and cooked once more.

**Cassava leaves:** Occasionally, cassava leaves accompanied *fuku* in Ubangi, *fufu* in Bas Zaire, and *bugali* in Kivu. In Ubangi, cassava leaves were quickly washed in cold water, ground in a wooden mortar, and boiled to obtain a spinach-like vegetable; palm oil, vegetable salt, and occasionally peanuts were added to produce *mpondu*. The same process was used in Kivu for *sombe*, which was only made with young leaves. *Sombe* was sometimes eaten with small fry. In Bas Zaire, cassava leaves were quickly washed in hot water, ground, and cooked in water for 1–2 hours; ground peanuts and, sometimes, fish were added.

**Grilled tubers:** *Bikedi* was a typical foodstuff in Bas Zaire. It was obtained from soaked bitter roots that were prepared in the same way as for cassava paste except that the soaked roots

were not cut into pieces but were grilled with oil.

## HCN content of cassava products

The HCN content of some of the cassava products prepared as described above were determined and are compared in Table 16. The scatter between the individual results was less than for fresh cassava roots and means could be calculated. As reported for fresh roots, cassava products from Bas Zaire had very low HCN content.

*Fuku* and *mpondu*, two typical food items from Ubangi, exhibited the highest values. In the Kivu area, *bugali* also contained appreciable amounts of HCN. As reported in chapter 2, *bugali* was prepared from cassava and sorghum grain, which also contains a cyanogenic glucoside (dhuririn) (Conn 1969). The HCN content of two samples of sorghum were

Table 16. HCN content of cassava products in Bas Zaire, Kivu, and Ubangi.

Food item	Number of samples	HCN content (mg HCN/kg)	
		Mean $\pm$ SEM	Range
Bas Zaire			
Soaked roots	3	2.1 $\pm$ 1.1	<1.0–4.2
<i>Fufu</i>	6	<1.0	<1.0–2.8
<i>Chickwangué</i>	8	1.3 $\pm$ 0.5	<1.0–4.8
Kivu			
Flour	17	21.6 $\pm$ 3.4	7.5–35.7
<i>Bugali</i>	19	6.3 $\pm$ 1.0	1.9–10.8
Ubangi			
<i>Fuku</i>	89	17.3 $\pm$ 1.1	3.1–22.6
<i>Mpondu</i>	22	8.2 $\pm$ 1.3	<1.0–25.0
<i>Fufu</i>	12	1.5 $\pm$ 0.4	<1.0–4.0
<i>Chickwangué</i>	17	2.7 $\pm$ 0.6	<1.0–7.2

Table 17. Relationship between detoxification processes of cassava roots and HCN content of the main cassava products consumed in Bas Zaire, Kivu, and Ubangi.

Food item	Detoxification process	Mean HCN content (mg/kg $\pm$ SEM)
Bas Zaire		
<i>Fufu</i>	Soaked twice and sun-dried	<1.0
Kivu		
<i>Bugali</i>	Fermented and sun-dried	6.3 $\pm$ 1.0
Ubangi		
<i>Fuku</i>	Sun-dried	17.3 $\pm$ 1.1

20.6 and 23.8 mg HCN/kg. Beans, another foodstuff widely eaten in Kivu, also contain linamarin (Dunstan and Henry 1903). The HCN content of dried beans collected in Kivu was  $8.4 \pm 7.5$  mg HCN/kg (mean  $\pm$  SEM; range, <1.0–15.9). Some other vegetables, for example, colocasses and green leaves, occasionally eaten by Kivu inhabitants, contained no measurable HCN.

The various detoxification processes for fresh cassava roots used in Ubangi, Kivu, and Bas Zaire and the resulting HCN content in the main food items eaten in these areas are shown in Table 17.

The lowest HCN content, in food from Bas Zaire, indicated that sequential soaking and sun-drying was apparently the most efficient detoxification process. Sun-drying alone, which is widely used in Ubangi, was less efficient and produced the food with the highest HCN content.

It must be emphasized that, because of the decrease in yield of cassava production and the food shortage now occurring in Bas Zaire, the local population has tended to shorten the period of soaking, particularly to avoid having roots stolen while they soak. If generalized, this reduced soaking might result in a progressive increase of the HCN content of the food in Bas Zaire as well.

## Study of detoxification processing

The large differences noted in the prepared food items collected from the three areas of Zaire led us to reinvestigate which step was essential or critical in the detoxification processes.

*Foodstuffs prepared in the laboratory at Gemena:* The main foodstuffs eaten in the Ubangi area were prepared by our chemists in the centre at Gemena using unselected samples of fresh cassava roots and leaves, bought at the local market. The food items were prepared according to the same procedures as those used by the local inhabitants. However, we particularly tried to detoxify the food as much as possible using the same procedures. For instance, some cassava leaves were cooked 15 min, as did Ubangi inhabitants, and others were cooked 30 min to evaluate a more effective detoxification. In the same way, *fufu* was prepared by adding a 3-day soaking period, which is exceptional in the Ubangi area.

The remaining HCN content after each step of the preparation in different foodstuffs is shown in Table 18. The food items prepared in the laboratory were very efficiently detoxified. For all of them, the final HCN content was about 1.0 mg HCN/kg. By contrast, six workers of the research centre were asked to pre-

Table 18. HCN content of various food items from Ubangi during preparation.

Food item <sup>a</sup>	Detoxification stage	Remaining HCN	
		Mean $\pm$ SEM (mg/kg)	%
<i>Mpondu</i> (6)	Fresh leaves	68.6 $\pm$ 22.9	100.0
	Washed leaves (cold water)	63.9 $\pm$ 19.2	93.1
	Dried leaves	66.1 $\pm$ 40.3	96.3
	Boiled leaves (15 min in water)	3.7 $\pm$ 2.2	5.4
	Boiled leaves (30 min in water)	1.2 $\pm$ 0.8	1.7
Boiled cassava (8)	Fresh roots (sweet)	10.7 $\pm$ 4.8	100.0
	Boiled roots (20 min in water)	1.3 $\pm$ 1.3	12.1
<i>Fufu</i> (12)	Fresh roots (sweet and bitter)	111.5 $\pm$ 90.3	100.0
	Soaked roots (3 days)	19.4 $\pm$ 23.5	17.4
	Dried roots (3 days)	15.7 $\pm$ 21.5	14.1
	Uncooked <i>fufu</i> (flour and water)	2.5 $\pm$ 1.6	2.2
	Cooked <i>fufu</i>	1.5 $\pm$ 1.4	1.3
<i>Fuku</i> (10)	Fresh roots (sweet)	25.5 $\pm$ 13.3	100.0
	Dried (1 day) and ground	193.6 $\pm$ 85.0	759.0
	Dried (2 days) and ground	54.3 $\pm$ 42.2	212.7
	Uncooked <i>fuku</i> (heated)	4.2 $\pm$ 5.5	16.4
	Cooked <i>fuku</i>	1.2 $\pm$ 1.2	4.7

<sup>a</sup>Numbers of preparations or roots shown in parentheses.

pare their own *mpondu* at the centre; the mean HCN content of their food items was  $10.0 \pm 10.5$  (SD) mg HCN/kg, with individual values in the range of  $<1.0$ – $25.0$  mg HCN/kg.

These results indicate that, even in the Ubangi area, well detoxified foodstuffs could be obtained from the same products as those used by local inhabitants if the detoxification processing is handled adequately.

*Drying and temperature:* While preparing *fuku*, we observed an increase in the HCN content of the roots dried for 1–2 days (Table 18). This experiment was repeated with 11 roots and gave similar results (Table 19).

In a subsequent experiment, the roots were dried for 1–8 days. When the water removed was expressed as a decrease in the initial weight of the roots (Table 20), it was clear that the longer the period of drying, the larger was the amount of water removed from the tubers.

Table 19. Effects of drying on the HCN content in 11 cassava roots.

Drying period (days)	Remaining HCN	
	Mean $\pm$ SD (mg/kg)	%
0	$70.4 \pm 53.0$	100.0
1	$95.5 \pm 65.9$	135.6
2	$91.1 \pm 89.6$	129.4
3	$56.6 \pm 43.8$	80.4

Clearly, from Tables 19 and 20, the main effect of drying was the removal of water from the roots. Consequently, a large part of the HCN remained in the roots and the apparent increase in HCN content resulted only from disappearance of water.

To examine the effects of heating on the HCN content of the roots, six cassava roots were divided into four identical parts (longitudinal section) and heated in an oven to constant weight (Table 21). Slight heating again produced an increase in the HCN content due to the loss of water. At  $105^\circ\text{C}$ , however, about 60% of the initial HCN content was lost and, at  $165^\circ\text{C}$ , almost all the HCN was released. These latter temperatures were chosen because they exceed the decomposition temperature reported for linamarase ( $72^\circ\text{C}$ ) (Joachim and Pandittesekere 1944) and linamarin ( $150^\circ\text{C}$ ) (Cerighelli 1955). Such temperatures, however, are never achieved by the Ubangi population while preparing their meals.

Table 20. Effects of drying on the percentage of water removed from six cassava roots.

Drying periods (days)	Mean percentage loss of water $\pm$ SEM
1	$14.1 \pm 3.2$
2	$51.1 \pm 2.5$
3	$61.1 \pm 0.6$
4	$64.5 \pm 2.0$
5	$68.9 \pm 4.3$
8	$70.0 \pm 5.3$

Table 21. Effects of heating on the HCN content of six cassava roots.

Part	Treatment	Mean HCN content (mg/kg $\pm$ SEM)
1	Fresh roots	$73.5 \pm 17.7$
2	Heated at $60^\circ\text{C}$	$116.7 \pm 15.3$
3	Heated at $105^\circ\text{C}$	$28.8 \pm 8.3$
4	Heated at $165^\circ\text{C}$	$<1.0$

*Effects of soaking:* To explore the effects of soaking during detoxification, the remaining HCN content of roots soaked for 1–5 days was measured. Soaking for only 1 day released 45% of the initial HCN content (Table 22) and soaking for 4 days decreased the HCN content by about 90%. Soaking for more than 5 days was tested but the roots decomposed entirely.

Because autolysis was used for the HCN determinations, the results obtained for HCN contents must be regarded as the lowest possible values. Indeed, if the linamarase was destroyed for any reason, autolysis could not produce HCN from persisting linamarin.

The hypothesis that linamarase could be destroyed by soaking was tested by adding fresh cassava roots with extremely low HCN con-

Table 22. Effects of soaking for 1–5 days on the HCN content of six bitter roots.

Soaking period (days)	Remaining HCN	
	Mean $\pm$ SEM (mg/kg)	%
0	$108.2 \pm 48.8$	100.0
1	$59.5 \pm 40.7$	55.0
2	$45.8 \pm 35.8$	42.3
3	$20.6 \pm 18.7$	19.0
4	$11.8 \pm 17.2$	10.9
5	$2.9 \pm 3.3$	2.7



tent, which supposedly contained the enzyme, to bitter roots after 6 days soaking. Inasmuch as sweet cassava did contain excess linamarase, the data in Table 23 showed that the low HCN content observed in the bitter roots after 6 days soaking did not result from deactivation or release of the enzyme, but was actually due to the release of the linamarin originally present.

Table 23. Effect of sweet cassava, as a possible source of enzyme, on the release of HCN in bitter soaked roots.

Root sample	Mean HCN content (mg/kg $\pm$ SEM)
Fresh sweet (I)	2.4 $\pm$ 0.2
Fresh bitter (II)	136.2 $\pm$ 9.4
Bitter soaked for 6 days (III)	1.4 $\pm$ 0.4
(I) + (III)	2.3 $\pm$ 0.5

### Trial of nutrition education in the Ubangi area

The trial of nutrition education was aimed at reducing SCN overload in humans by modifying their food habits. The investigation was carried out in the village of Bokuda, 25 km from Gemena. A family of 29 persons was asked to modify the preparation of their usual food (mainly *fuku*) by adding a 3-day period of soaking, 1 day drying of the soaked roots, and cooking the flour for 30 min. The reasons for these proposals were explained at length orally and with pictures. The formal consent of the whole family was obtained thanks to the very good relations between the research team and the head of the family, who was also the head (*capita*) of the village. Urine samples were collected on days 0, 7, 14, 21, 28, 42, and 49 for SCN measurement.

The mean urinary SCN concentrations obtained before the modification of processing (on day 15) were fairly constant and close to 1 mg/dl (Fig. 11). After the modification, there was a slight but not significant decrease in the mean urinary SCN concentration on days 21 and 28. Surprisingly, at days 42 and 49, the results were similar to or even higher than the initial values. All subjects exhibited a similar trend. The results were shown to the villagers and, on questioning, they explained that they had followed the protocol for only 3 days and after that had preferred to sell well detoxified

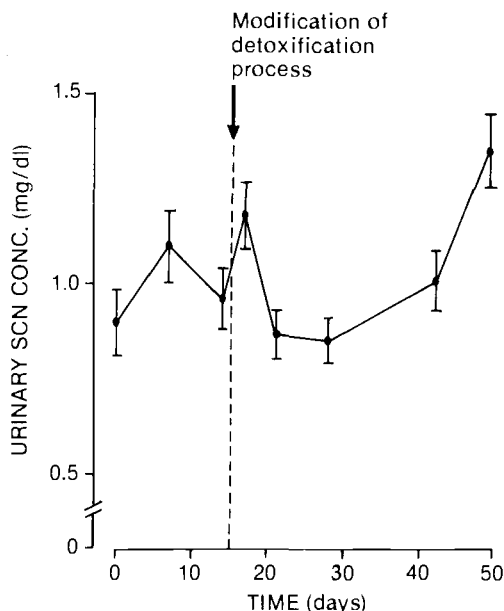


Fig. 11. Changes with time in urinary SCN concentrations (mean  $\pm$  SEM) in 29 persons in the village of Bokuda (Ubangi) during a trial of nutrition education.

cassava, i.e., *chickwangue*, at the market of Gemena to get some money.

This unsuccessful trial showed that, even with carefully prepared and apparently motivated people, changing the nutritional habits of a rural population is quite difficult.

### Discussion

The HCN content of food items consumed in the three areas investigated decreases from Ubangi through Kivu to Bas Zaire. These variations reflect both a difference in the HCN content of fresh roots and, especially, the efficiency of the detoxification processes used in the three areas. The HCN content of fresh roots showed a wide scatter but, on the whole, the results seem to indicate that HCN content of the roots decreases from Ubangi through Kivu to Bas Zaire. Since no botanical determination or soil analysis could be performed, our data do not allow us to conclude whether the different HCN contents of roots are related to genetic or environmental factors, or both. However, we observed that cassava varieties growing in the Kivu area are quite different from those cultivated in Ubangi and Bas Zaire.

In the Ubangi area, cassava is most usually dried in the sun and soaking is rather excep-

tional. Samples of the main food eaten by the inhabitants, i.e., *fuku*, contain an average of 17 mg HCN/kg. The high HCN content observed in *fuku* is closely related to the detoxification process used in that area. Indeed, experimental studies show that sun-drying of cassava is an inefficient process of detoxification. As indicated in Tables 19–21, sun-drying of roots results mostly in a loss of water rather than release of HCN. During the preparation of *fuku*, we observed that the critical step of detoxification occurs while heating or boiling cassava flour. Studying the effects of heating, we observed that the temperature required for complete release of HCN from the roots (i.e., more than 150°C) is never reached during sun-drying or preparation of the meals.

Despite the efficient release of HCN noted in food items prepared in the laboratory (up to 95% of the initial HCN content), the traditional way of preparation of foodstuffs by the inhabitants results only in a partial release (about 80%).

In contrast, in Bas Zaire, soaking is universally used and detoxifies the roots efficiently. The reduction of HCN ranges from 45% after soaking for only 1 day to 90% after 4 days. The latter value is of considerable interest. Processing that includes sequential soaking (twice whenever possible), sun-drying, and cooking results in virtually complete release of HCN, as is shown by the very low HCN content in food from Bas Zaire.

In the Kivu area, detoxification processing that includes sun-drying and fermentation appears to be fairly efficient since the HCN content of foodstuffs is lower than in Ubangi but slightly higher than in Bas Zaire.

The apparently conflicting observation that the HCN content of foodstuffs is higher in Kivu than in Bas Zaire while serum and urinary SCN concentrations in humans are practically similar in both areas (see chapters 2–4) may be partly explained by seasonal variations in the consumption of processed cassava. In Kivu, as reported earlier, cassava is only eaten

from July to November. When considering separately the group of 58 adults investigated in Kivu during July and August, when the food samples were collected, serum and urinary SCN concentrations were  $1.10 \pm 0.07$  mg/dl and  $2.59 \pm 0.31$  (SEM) mg/dl, respectively, i.e., values higher than those reported for adults in Bas Zaire or for adults in Kivu investigated between January and June. The role played by seasonal variations in the consumption of cassava in Kivu could not be further explored.

An attempt to reduce the SCN overload in apparently motivated inhabitants in the Ubangi area using nutrition education failed entirely. This underlines the well recognized difficulty of modifying the food habits of rural populations in Africa. Such an attempt requires a more sophisticated approach based on an accurate knowledge of the psycho-socioeconomic context of these populations.

Finally, we must point out that the nutritional value of cassava is reduced when it is processed (Longe 1980). In particular, Rajaguru (1975) has reported that soaking removes the soluble proteins.

In conclusion, the data reported indicate that the differences in the HCN content of cassava products eaten by the local populations of Ubangi, Kivu, and Bas Zaire are closely linked both to differences in the content of HCN of fresh roots and to the regional variations in traditional cassava processing. In this context, soaking may be regarded as the most efficient detoxification process. The differences in the HCN content of food items may account for the variations in the SCN levels observed in humans in the three areas investigated in Zaire.

From the available data, it can be expected that, if for any reason including food shortage due to socioeconomic conditions, the dietary supply of cassava increased or the efficiency of the detoxification process decreased, cassava toxicity for the thyroid in humans would become evident in areas that are now unaffected.



## Chapter 6

# Breast Feeding, Thiocyanate Metabolism, and Thyroid Function in Young Infants in Severe Endemic Goitre

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**Allaitement maternel, métabolisme du thiocyanate et fonction thyroïdienne chez le jeune enfant en région de goitre endémique sévère — Résumé —** Des données antérieures avaient montré que dans l'endémie goitreuse de l'Ubangi, près de 10 % des nouveau-nés présentaient un tableau biochimique d'insuffisance thyroïdienne et que le SCN d'origine maternelle jouait un rôle déterminant dans le déclenchement de cette hypothyroïdie. La question se posait de savoir dans quelle mesure les conditions nutritionnelles présentes immédiatement après la naissance et au cours des premières années de la vie affectent la fonction thyroïdienne du jeune enfant. Une attention particulière a été apportée aux suppléments

au lait maternel administrés aux enfants allaités étant donné que nous avons observé antérieurement que ces derniers avaient des concentrations sériques de SCN élevées alors que le contenu en SCN du lait maternel dans l'Ubangi n'était pas plus élevé qu'à Bruxelles.

L'étude a porté sur 311 enfants âgés de 0,2 à 83 mois et a consisté à apprécier l'évolution en fonction de l'âge, de la prévalence du goitre, des concentrations urinaires en SCN et en iode et des concentrations sériques en SCN, TSH,  $T_4$  et  $T_3$ . Elle a comporté également l'appréciation, en fonction de l'âge, de l'importance relative de l'allaitement maternel et de suppléments alimentaires administrés aux enfants.

De la naissance jusqu'à 84 mois, la carence iodée est très importante et ne varie pas en fonction de l'âge (iode urinaire : 1,7 à 2,2  $\mu\text{g}/\text{dl}$ ). Par contre, le SCN sérique, élevé à la naissance (0,75  $\text{mg}/\text{dl}$ ), diminue progressivement au cours de la première année jusqu'à environ 50 % de sa valeur initiale. Mais à partir de l'âge de 36 mois, il remonte pour atteindre les taux rencontrés chez l'adulte. La prévalence du goitre augmente également en fonction de l'âge, parallèlement à l'élévation du SCN sérique (Fig. 12).

L'augmentation simultanée de ces deux variables correspond à l'introduction progressive de suppléments au lait maternel (Fig. 13), essentiellement sous forme de jus de manioc non roui. Ces suppléments apparaissent dès l'âge de 6 semaines et sont observés chez 50 % des enfants de 4 mois. Le sevrage est terminé chez 50 % des enfants de 27 mois et chez 100 % à l'âge de 60 mois. Les concentrations sériques de TSH obtenues pour l'ensemble des enfants étudiés sont en moyenne plus élevées que chez des témoins belges du même âge et celles de  $T_4$  sont plus basses. À partir de l'âge de 60 mois, soit au moment où tous les enfants sont sevrés, on observe une élévation supplémentaire de la TSH (Fig. 14, Tableau 24). Les concentrations sériques de  $T_4$  sont directement corrélées à l'iode urinaire et inversement corrélées au SCN urinaire (Tableau 25).

En conclusion, ce travail montre que la surcharge en SCN observée chez le jeune enfant en Ubangi résulte de l'introduction précoce de suppléments au lait maternel sous forme de dérivés du manioc et que cette surcharge joue un rôle déterminant dans le développement du goitre et du dérèglement de la fonction thyroïdienne observé durant la période critique du développement cérébral. L'allaitement maternel semble donc jouer un rôle protecteur vis-à-vis du crétinisme endémique dans l'Ubangi, essentiellement en raison du délai qu'il entraîne dans l'introduction de suppléments de manioc dans l'alimentation du jeune enfant.

The high values of SCN concentrations in serum from cord blood reported in Ubangi result from the transfer of SCN across the placenta (Delange et al. 1980a; see also chapter 4). The results reported in chapter 4 also show that the SCN overload in fetal life plays a determining role in the etiology of the biochemical picture of congenital hypothyroidism found in about 10% of the newborns in this region (Thilly et al. 1978, 1980a).

The question arises as to what extent the nutritional conditions during the first years of life in Ubangi affect the iodine and SCN dietary supplies of young infants and how the thyroid adapts itself to this new environment.

SCN is not concentrated by the mammary gland in humans (Funderburk and Van Middlesworth 1967) and, consequently, its level in maternal milk is low, even in areas where the HCN supply to lactating mothers is markedly elevated (Delange et al. 1980a). One would expect therefore that, during breast feeding, infants should have normal SCN serum levels in Ubangi. Conversely, rather elevated values ( $0.55 \pm 0.04$  (SEM) mg/dl) were found in a group of 54 infants aged 2 weeks to 15 months in this area (Delange et al. 1980a).

The objective of the present investigations were:

- To follow the change in concentrations of serum and urinary SCN during the first years of life in Zairian infants, particularly during the period of breast feeding.
- To assess the relationship between these concentrations and the characteristics of the diet.
- To further assess thyroid function in young infants and children in Zaire, especially during the first 3 years of life, the critical period of brain development.
- To determine the role played by SCN overload during this critical period on the development of goitre and on thyroid function.

## Patients and Methods

The study was carried out at the Hospital of Karawa, in a part of the Ubangi endemic goitre area where the iodine deficiency has not yet been corrected by injections of iodized oil to the general population.

It consisted of a clinical and biochemical assessment of thyroid function and SCN metabolism in infants and children and of a survey of the mothers to determine the children's diets.

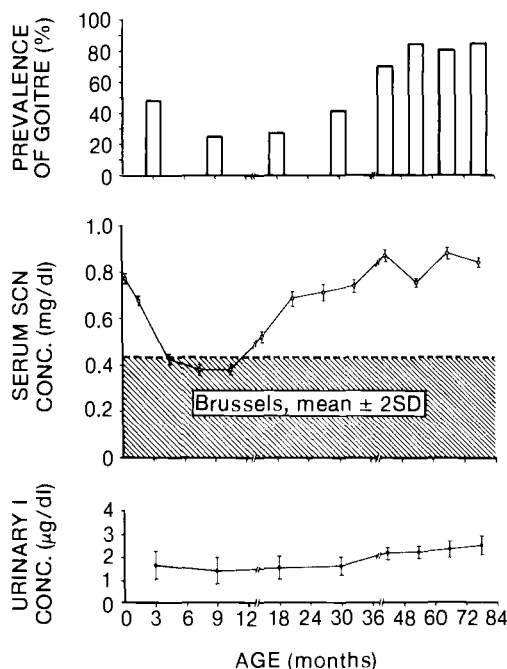
The study included 311 patients aged 0.2–83 months (see Table 24) born to untreated pregnant women. They were examined clinically, including description of the volume of the thyroid gland, and blood and urine samples were collected for determination of the concentrations of serum SCN, TSH,  $T_4$ , and  $T_3$  and urinary iodine and SCN, and calculation of the urinary I/SCN ratios.

The dietary survey of the mothers focused mainly on the breast-feeding habits during the first 5 years of the infant's life. Each mother was asked whether her infant was exclusively breast fed, breast fed and supplemented, or weaned. The nature of the supplement to breast milk, if any, was carefully noted, including the type of food used as supplement and its preparation before administration to the infant. Since quantities of breast milk and supplement were not weighed, the nutrition survey was only qualitative. The results were expressed by the frequency distributions of patients exclusively breast fed, breast fed and supplemented, and weaned as a function of age. No chemical assays were performed on breast milk or the dietary supplements.

## Results

The changes with age in the concentrations of urinary iodine and serum SCN, and in the prevalence of goitre in the 311 patients are shown in Fig. 12. The urinary concentrations of iodine varied from 1.7 to 2.2  $\mu\text{g}/\text{dl}$  and did not show any significant change with age. They were lower ( $P < 0.001$ ), however, than the control value from 127 Belgian infants of the same age ( $5.4 \pm 0.3$  (SEM)  $\mu\text{g}/\text{dl}$ ). These results indicate that, during the whole study period, all the patients suffered from severe, uniform iodine deficiency.

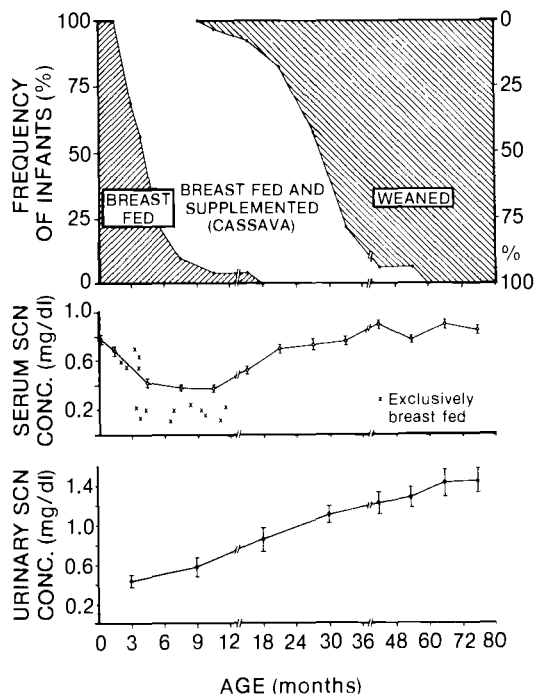
In contrast, the serum SCN concentrations varied markedly with age. The mean value observed for cord blood from 171 newborns in the same area was  $0.75 \pm 0.03$  (SEM) mg/dl. During the first 6 months of life, the concentrations decreased linearly to reach 50% of the value observed at birth by 5.2 months. During the second semester of life, the SCN concentrations remained almost unchanged near the upper limit of normal for the Belgian controls of the same age. From the age of 12 months, the concentrations increased progressively to reach a maximum value of about 0.8 mg/dl at the age of 36 months. This value corresponds to that observed in adults in the same area.



**Fig. 12.** Changes in concentrations of urinary iodine and serum SCN, and in prevalence of goitre as a function of age in the Ubangi area. (Mean  $\pm$  SEM. Shaded area corresponds to the normal range (mean  $\pm$  2 SD) in euthyroid Belgian infants.)

The prevalence of goitre also varied greatly with age and the shape of the curve paralleled that for the serum SCN concentrations. The prevalence was 49% during the first semester of life, reached a minimum of 25% during the second semester ( $\chi^2 = 9.62$ ,  $P < 0.001$ ) and then increased progressively to reach a plateau of about 75% from the age of 36 months.

The results of the nutrition survey are shown in Fig. 13, which compares the characteristics of the dietary intake of the patients to concentrations of serum and urinary SCN. Breast milk was the sole nutritional supply in all infants (100%) only up to the age of 6 weeks. From that age, the frequency of supplemented infants increased rapidly and reached 50% at the age of 4 months. In all infants, the first supplement consisted of a juice obtained from unsoaked cassava mixed with corn. Later, it consisted of a more solid paste, also made from unsoaked cassava as in the adult diet (*fuku*), with beef and fish. The age at weaning varied from 9 to 60 months; 50% of the infants were weaned by the age of 27 months. At weaning, the nutritional characteristics of the diet were



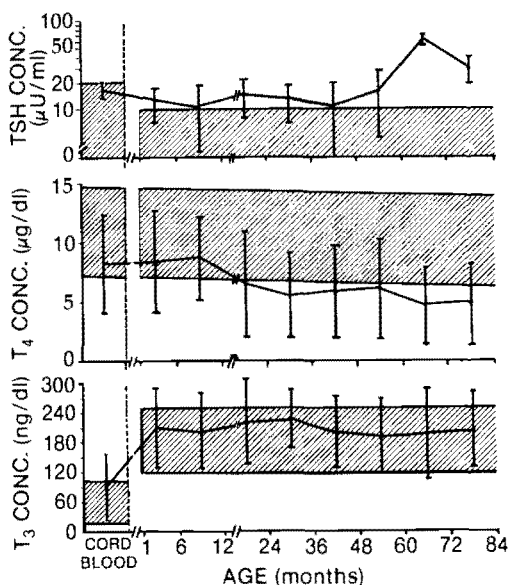
**Fig. 13.** Results of the nutritional survey and comparison of changes with age in concentrations of serum and urinary SCN. (Mean  $\pm$  SEM.)

similar to those of adults, being based mainly on cassava.

The progressive decrease of the serum SCN during the first semester of life corresponded to the period during which breast milk constituted the main dietary supply and its subsequent increase corresponded to the increasing frequency of diet supplementation with cassava juice. However, mean serum SCN clearly increased only when more than 95% of the infants were supplemented with cassava.

The concentrations of urinary SCN increased progressively with age and reached a maximum value of 1.4 mg/dl at the age of 72 months. From the age of 9 months onwards, urinary and serum SCN concentrations were directly related whereas, during the first semester of life, the urinary concentration was only 0.4 mg/dl, i.e., a minimally elevated value, in spite of significantly elevated serum levels.

The change in levels of serum TSH,  $T_4$ , and  $T_3$  in the same patients is shown in Fig. 14, as are the values observed in cord blood in the area. The geometric means of the serum TSH levels were systematically higher than the up-



**Fig. 14.** Changes in levels of serum TSH,  $T_4$ , and  $T_3$  from birth to age 7 years. (Arithmetic mean  $\pm 1$  SD for  $T_4$  and  $T_3$ ; geometric mean  $\pm 1$  SD for TSH. Shaded area corresponds to the normal range (mean  $\pm 2$  SD) in euthyroid Belgian infants.)

per limit of normal for age during the whole study period. Only 47% of the 311 patients had TSH values situated within the normal range ( $<10 \mu\text{U/ml}$ ) and 27% had values above  $100 \mu\text{U/ml}$  (Table 24). These extremely elevated values were observed in 17–24% of the infants age 0–47 months and this frequency increased to 56% by the age of 60 months.

The mean serum  $T_4$  concentrations (Fig. 14) were at the lower limit of normal from birth to

**Table 24.** Frequency distribution of the serum levels of TSH ( $\mu\text{U/ml}$ ) as a function of age ( $<10$ , normal;  $\geq 25$ , elevated, clearly abnormal;  $\geq 100$ , extremely elevated, indicating thyroid failure).

Age groups (months)	Number of patients	Frequency (%) of patients with serum TSH ( $\mu\text{U/ml}$ ) of:		
		$<10$	$\geq 25$	$\geq 100$
0–5	36	50	39	19
6–11	49	61	31	24
12–23	29	48	38	17
24–35	41	49	32	17
36–47	47	51	36	17
48–59	51	49	39	31
60–71	34	20	70	56
72–83	24	33	58	42
Total	311	47	41	27

the age of 12 months and were systematically lower than this limit after the age of 24 months. In contrast, the mean serum  $T_3$  concentrations were at the upper limit of normal from birth to the age of 84 months.

When correlation coefficients were calculated between serum  $T_4$  on the one hand and age, serum TSH and SCN, urinary iodine and SCN, and urinary I/SCN ratio on the other (Table 25), it was clear that the serum  $T_4$  levels were related to the concentrations of urinary iodine directly and to urinary SCN indirectly. The correlation with the urinary I/SCN ratio was not higher than with the urinary iodine concentrations.

**Table 25.** Correlation coefficients for 104 patients between serum  $T_4$  (first variable) and age, serum TSH and SCN, and urinary I, SCN, and I/SCN ratio (second variable).

Second variable	Correlation coefficients (r)	Significance level (P)
Age	-0.46	$<0.001$
Serum TSH	-0.68	$<0.001$
Serum SCN	-0.18	NS
Urinary I (log)	+0.24	$<0.05$
Urinary SCN	-0.34	$<0.05$
Urinary I/SCN ratio	+0.22	$<0.05$

## Discussion

This work confirms that young infants in the Ubangi endemic goitre area already have elevated levels of serum SCN and frequently exhibit a biochemical profile of severe thyroid failure. It shows that the main mechanism responsible for SCN overload in these infants is the early introduction of supplements to breast feeding that consist essentially of juice of unsoaked cassava. It also shows that this SCN overload plays a critical role in the development of goitre and of thyroid failure during the first years of life.

The levels of serum SCN observed in young infants depend on three factors: its concentration at birth; its disappearance rate from the serum resulting mainly from its urinary excretion, which is the major route of elimination (Funderburk and Van Middlesworth 1971; Kreutler et al. 1978; Ermans et al. 1980a); and its endogenous production, which in this area is mainly related to cassava ingestion.

In Ubangi, serum SCN concentrations in cord blood are extremely elevated due to its placental transfer from the mothers (Delange

et al. 1980a). However, this observation could not account for the high levels still observed several months after birth if the half-life of SCN in newborns is similar to that of 4–10 days in normal adults (Eder 1951; Pettigrew and Fell 1972; Butts et al. 1974; Bourdoux et al. 1978; Schulz et al. 1979). During the first semester of life, the concentration of urinary SCN is only slightly elevated while in serum it is markedly elevated. This situation contrasts with the observation made in older children and in adults in whom the concentration of urinary SCN is directly related to its serum concentration (Bourdoux et al. 1978, 1980b). This discrepancy could be explained by renal immaturity in young infants resulting in a lower clearance rate of SCN than in children and in adults. This lower clearance could, in turn, partly explain the much longer biological half-life of SCN in young infants than in adults.

This work shows that breast milk is the sole dietary supply of young infants only up to the age of 6 weeks. Subsequently, supplements are rapidly introduced, consisting primarily of cassava juice, probably with a high content of HCN. Therefore, elevated serum SCN observed in partly breast-fed young infants could result from its high level at birth, renal immaturity, and endogenous formation of SCN from cassava. The role played by the last factor is suggested by the observation that exclusively breast-fed infants aged 6–12 months (Fig. 13) have a serum SCN much lower than the mean value observed in the general population of infants of the same age.

Although quantitative data on the dietary supplements to young infants during breast feeding and on the HCN content of these supplements are lacking, some conclusions can be drawn. The increasing frequency of introduction of supplements and of weaning parallels the increase of serum SCN levels; this indicates that the higher levels of serum SCN observed in young infants in Ubangi, compared with Belgian controls, result from early introduction of cassava to the diet of the infants.

Moreover, the parallel between the increase in serum SCN concentrations and the prevalence of goitre from birth to the age of 7 years in patients with severe, uniform iodine deficiency strongly suggests that SCN plays a determining role in the development of goitre from this early period of life, a well established fact in adults (Bourdoux et al. 1980a; Ermans et al. 1980a).

This work also shows that the previously reported high frequency of biochemical features of severe thyroid failure in newborns is also present in young infants. From birth to the age of 3 years, that is during the critical period of brain development (review by Delange 1981), about 20% of the infants have a serum TSH level higher than 100  $\mu$ U/ml and a  $T_4$  level lower than the mean minus 2 SD for normal Belgian controls. According to the criteria used in programs of neonatal screening for congenital hypothyroidism in Western countries (Delange et al. 1980c; Dussault et al. 1980), these biochemical indices strongly suggest congenital hypothyroidism, which is encountered in 1 in 4000 newborns (Burrow and Dussault 1980), i.e., an incidence of 0.025% live births. It thus appears that the incidence of congenital hypothyroidism with possible irreversible brain damage could be 1000 times higher in the Ubangi area than in Western countries. This probably represents the major public health problem for the affected African population.

The direct relationship between the levels of serum SCN and the prevalence of goitre and especially the indirect correlation between urinary SCN and  $T_4$  provide additional evidence that SCN does play a determining role in the development of thyroid failure and thus of myxedematous endemic cretinism in this particular population.

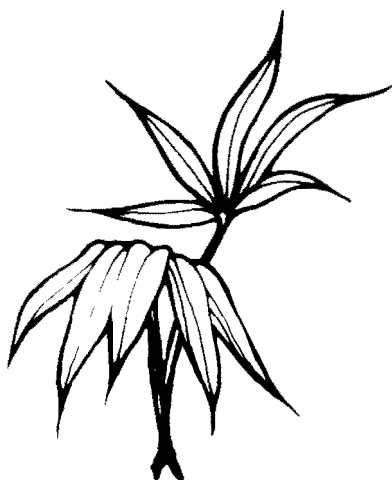
Breast feeding appears to play a protective role against the development of cretinism in the Ubangi population. This effect could possibly be due to the presence in human milk of thyroid hormones, in particular  $T_4$  or iodine, or both (Bode et al. 1978; Varma et al. 1978; Hemken 1980; Sack et al. 1980). Although  $T_4$  and iodine were not measured in human milk from the lactating mothers in the Ubangi area, unpublished data collected in our laboratory by Dr J. Varea Teran and Mrs J. Golstein-Golaire indicate that the  $T_4$  content of human milk is extremely low and that the much higher values mentioned above could be methodological artifacts. The protective action does not seem to result from a supplement of iodine contained in the milk, as the degree of iodine deficiency is similar in breast-fed and weaned infants. The major protective effect of breast feeding in African young infants thus appears to be related to the delay in the introduction of cassava to the diet.

In summary, this work shows that the overload of SCN observed in young infants in the

Ubangi area results from the early introduction of a cassava-based diet as supplement to breast feeding and that this SCN overload plays a determining role in the development of goitre, thyroid failure, and thus cretinism. Apart from the correction of iodine deficiency, a prophylactic measure could be to extend the period of

lactation as much as possible. This is another reason for strongly supporting breast feeding in developing countries. Another preventive measure could be to improve the detoxification process of cassava used as supplement to breast-fed infants. Such a program is now being conducted in the Ubangi area.





## Chapter 7

# Role of the Balance Between the Dietary Supplies of Iodine and Thiocyanate in the Etiology of Endemic Goitre in the Ubangi Area

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F. DELANGE

## Rôle de la balance entre les apports alimentaires en iode et en thiocyanate dans l'étiologie du goitre endémique en Ubangi

— **Résumé** — La situation épidémiologique observée le long de deux routes situées à la limite sud de l'endémie goitreuse de l'Ubangi (Fig. 15) nous a permis de préciser davantage le rôle respectif joué par le SCN et par la carence iodée dans l'étiologie du goitre dans cette région. Dans les 49 villages répartis sur les deux routes longues d'environ 100 km chacune, la prévalence du goitre dans la population (23 578 habitants) augmente progressivement de 20 à 80 %. Du crétinisme endémique survient lorsque la prévalence du goitre dépasse le seuil critique de 50 % de la population (Fig. 16). Des études biochimiques ont été effectuées chez 257 adultes cliniquement euthyroïdiens habitant 10 villages échelonnés sur les deux routes. Le long

de chacune d'elles, les taux moyens de TSH et de  $T_4$  sont situés dans les limites de la normale aussi longtemps que la prévalence de goitre est inférieure à 60 % de la population. Au-dessus de ce seuil critique, la TSH s'élève brusquement ( $P < 0,01$ ) et la  $T_4$  s'abaisse ( $P < 0,01$ ) (Fig. 17). Le long d'une des routes (axe ouest-est), la concentration de l'iode urinaire est très basse (1,7 à 2,5  $\mu\text{g}/\text{dl}$ ) et demeure pratiquement constante. Par contre, le SCN urinaire s'élève de 0,7 à 3,2  $\text{mg}/\text{dl}$  ( $P < 0,001$ ), suite à une augmentation progressive de la consommation de manioc non roui. Le long de l'autre route (axe sud-nord), le SCN urinaire demeure constant aux environs de 1,5  $\text{mg}/\text{dl}$  tandis que l'iode urinaire diminue progressivement en raison de la diminution de consommation de poisson. En conséquence, le long des deux routes, le rapport urinaire I/SCN tombe de 3,1 dans les villages où la prévalence du goitre est basse à 0,70 et 0,75 dans les villages hyperendémiques (Fig. 18).

En conclusion, l'apparition de goitre et de crétinisme endémiques dans cette région dépend de la balance entre les apports alimentaires en iode et en SCN plutôt que de l'apport absolu en iode. Le rôle spécifique joué par le SCN dans l'étiologie du goitre est démontré par l'apparition de goitre suite à une augmentation de l'apport en SCN en présence d'une carence iodée uniforme.

Although the goitrogenic effect of cassava is well documented in animals (Ekpechi et al. 1966; Ekpechi 1967, 1973; Ermans et al. 1972, 1973, 1980b; Delange et al. 1973; Maner and Gomez 1973; Van Der Velden et al. 1973; Tewe 1976), the precise role of this food when consumed by humans remains difficult to demonstrate for three reasons:

- An iodine deficiency is observed in the goitrous regions where the role of cassava has been suggested.
- The high serum levels of SCN observed in the inhabitants of these regions are insufficient to inhibit the uptake of radioiodine by the thyroid.
- Experimentally, impairment of thyroid function from prolonged ingestion of cassava is similar to that induced by iodine deficiency.

However, one of our previous observations in Ubangi (Ermans et al. 1980a) strongly suggested the role played by cassava: a small community living in a zone of extremely severe endemic goitre and temporarily subjected to a restriction of cassava showed partial nor-

malization of the serum levels of TSH and  $T_4$ , whereas the degree of iodine deficiency remained unchanged (Bourdoux et al. 1978, 1980b).

The purpose of the work reported in this chapter was to reevaluate the influence of the consumption of cassava on the etiology of endemic goitre by comparing the relative effects of different levels of iodine and SCN in the diet on the prevalence of goitre and on various variables of thyroid function. This study was made possible by the peculiar epidemiological situation observed in the Kungu zone in north-western Zaire (Fig. 15). In this zone, situated at the southern limit of the Ubangi endemic goitre area, a preliminary study had shown important variations in the prevalence of goitre among the communities located along two roads, each of which was about 100 km long.

Particular attention was paid to the dietary habits of these communities and to the HCN content of foods prepared from cassava.

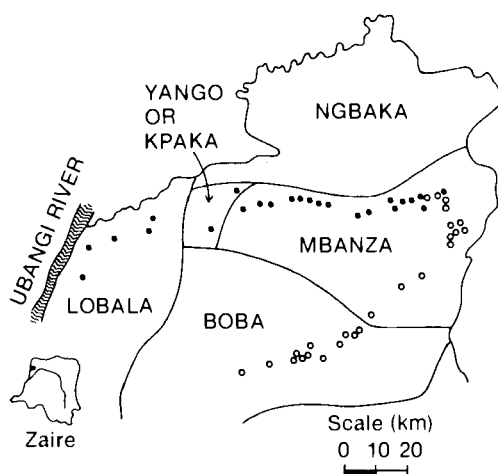


Fig. 15. Geographic and ethnographic distribution of Kungu villages studied along the West-East (closed circles) and South-North (open circles) roads.

## Patients and Methods

The Kungu zone is inhabited by Sudanese and Bantu ethnic groups who live in a self-subsistence economy. The Sudanese (Mbanza, Ngbaka, and Yango) are large consumers of unsoaked cassava, whereas the Bantu (Lobala and Boba) eat bananas, fish, and bread from soaked cassava (Simons-Gerard et al. 1980).

We determined the prevalence of goitre and endemic cretinism along the two road axes indicated in Fig. 15. The study was made on a total of 23 578 inhabitants distributed among the 49 villages on the two road axes, or 80% of the total population counted by the Zairian administration.

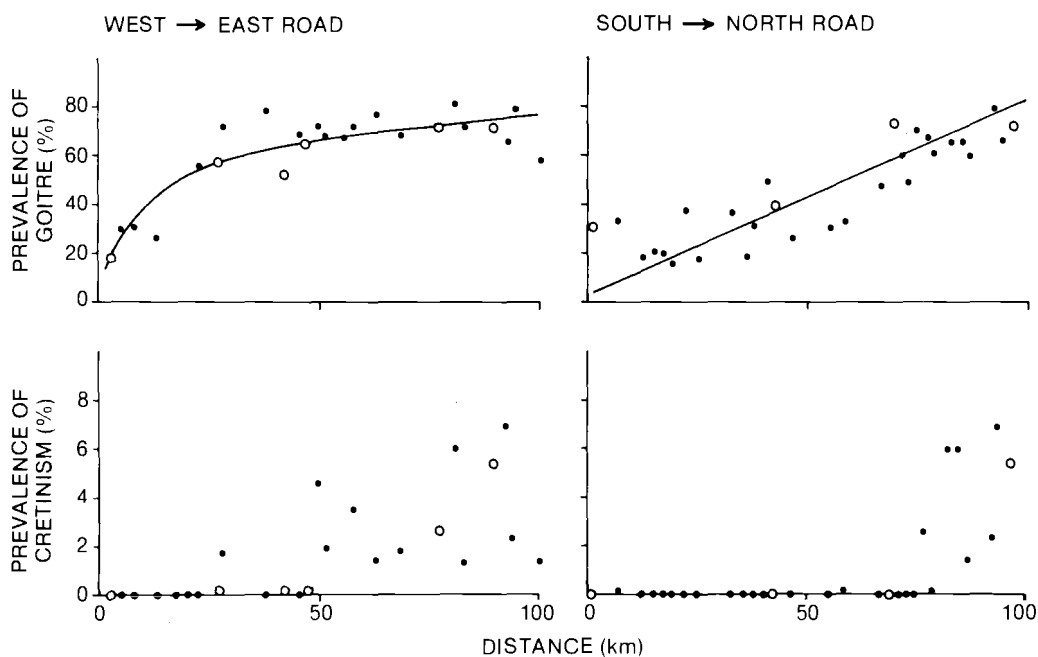
In 10 villages located along the two roads (indicated by open circles in Fig. 16), about 30 men and women per village, aged 15–35 years, were selected at random for metabolic studies to determine their concentrations of serum TSH,  $T_4$ ,  $T_3$ , and SCN and of urinary iodine and SCN. In addition, in seven villages along the West-East road, we questioned 50 women per village on dietary habits. This was a qualitative nutritional survey aimed at determining how often during the day the principal foods were consumed, particularly those prepared from cassava — gruel of cassava flour mixed with maize (*fuku*), ground boiled cassava leaves (*mpondu*), and boiled cassava roots soaked and mashed into a puree (*chickwangu*).

## Results

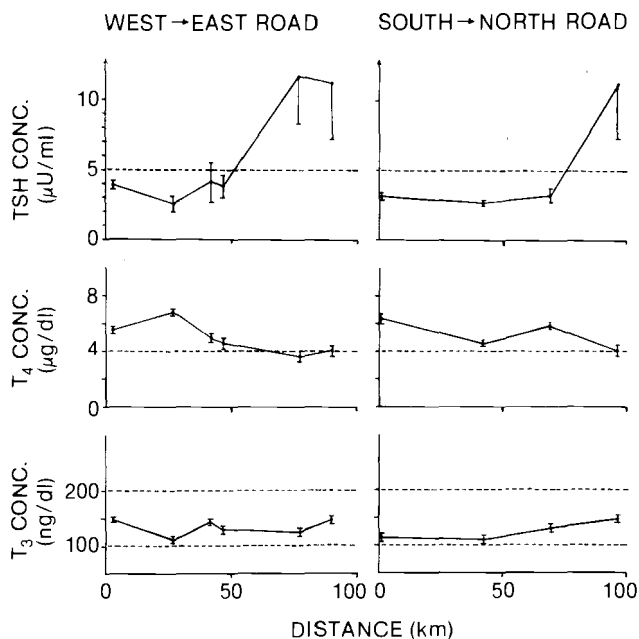
The prevalences of goitre and cretinism observed along the West-East and South-North roads in the Kungu zone are shown in Fig. 16. Goitre increased progressively from about 20 to 80% of the total population towards the east or towards the north. For the West-East road, this progression was very rapid over the first few kilometres, then very slow. For the South-North road, the progression was linear along the road. On the two roads, myxedematous endemic cretinism increased progressively when the prevalence of goitre reached the critical threshold of about 50% of the total population.

The concentrations of serum TSH,  $T_4$ , and  $T_3$  observed in the villages where metabolic studies were carried out are shown in Fig. 17. Serum TSH values were within the normal limits where the prevalences of goitre were lower than 60% — in the first 50 km on the West-East road and the first 75 km on the South-North road (Fig. 16). Above this value, it increased sharply to higher levels ( $P < 0.01$ ). This modification was accompanied by a reduction in  $T_4$  values ( $P < 0.05$ – $0.01$ ) whereas  $T_3$  values were not different ( $P > 0.05$ ). The change in levels of serum TSH,  $T_4$ , and  $T_3$  was almost similar along the two roads studied.

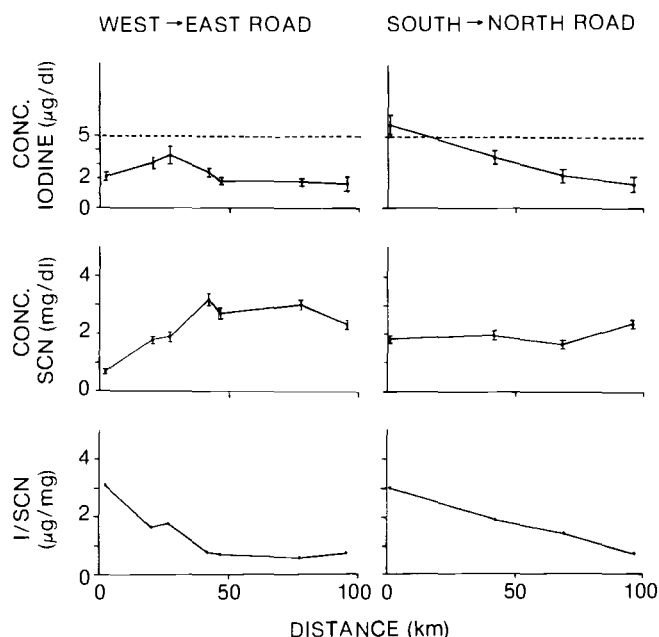
The concentrations of urinary iodine and SCN and the urinary I/SCN ratios in the same



**Fig. 16.** Changes in prevalence of goitre and cretinism along the two roads investigated. (Each point represents one village: open circles represent those where metabolic investigations were carried out.)



**Fig. 17.** Changes in concentrations of serum TSH,  $T_4$ , and  $T_3$  in young adults along the two roads studied (Mean  $\pm$  SEM. Dotted lines represent limits of normal for Belgian adults.)



**Fig. 18.** Changes in concentrations of urinary iodine and SCN and in urinary I/SCN ratios along the two roads. (Mean  $\pm$  SEM. Dotted lines represent limit of normal for Belgian adults.)

villages are shown in Fig. 18. Along the West-East road, the mean concentrations of iodine varied from 1.7 to 2.5  $\mu\text{g/dl}$ . The levels remained practically unchanged along the road in spite of a progressive increase in the prevalence of goitre. However, these values were significantly lower than in normal Belgian adults used as controls. The concentrations of SCN, which were low in the west, increased progressively to the east, paralleling the increase in prevalence of goitre.

In contrast, along the South-North road, the concentration of urinary iodine was normal (6  $\mu\text{g/dl}$ ) in the south but diminished progressively ( $P < 0.01$ ) to 2  $\mu\text{g/dl}$  in the north (Fig. 18). The concentrations of urinary SCN were high and constant all along the road.

Along the two roads, the urinary I/SCN ratios diminished progressively as the prevalence of goitre increased. The change was similar along the two roads. The ratios were 3.1 and 3.0 in the villages with a low prevalence of goitre, and 0.75 and 0.70 in the hyperendemic villages. These differences were highly significant ( $P < 0.01$ ).

The correlation coefficients between the prevalence of goitre and the concentrations of urinary iodine and SCN and the I/SCN ratios

are shown in Table 26. Along the West-East road, prevalence of goitre was highly significantly correlated with the concentration of urinary SCN, but not with urinary iodine. The opposite situation was observed along the South-North road. For both roads, the correlations between the prevalence of goitre and the urinary I/SCN ratio were practically identical. It was, however, significant only for the West-East road, probably because of the small sample size.

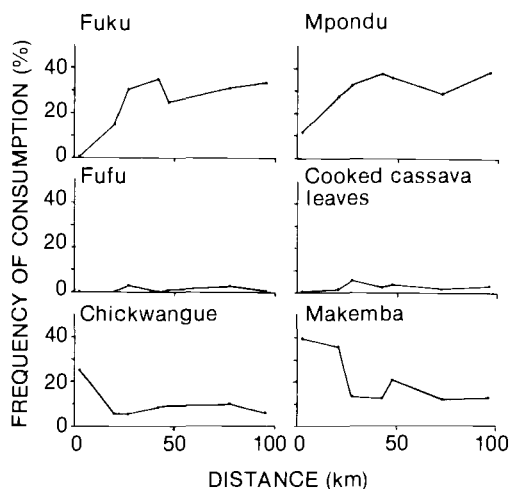
Frequency of consumption of the principal foods changed along the West-East road

**Table 26.** Correlation coefficients<sup>a</sup> between the prevalence of goitre (first variable) and the concentrations of urinary iodine and SCN and the urinary I/SCN ratio (second variable) for the two roads.

Second variable	West-East road (6) <sup>b</sup>	South-North road (4)
Urinary SCN	0.9545***	0.2628NS
Urinary iodine	-0.2030NS	-0.9670**
I/SCN ratio	-0.8902**	-0.8397NS

<sup>a</sup>Levels of significance: NS, not significant; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

<sup>b</sup>Values in parentheses are numbers of villages sampled.



**Fig. 19.** Results of nutritional survey along the West-East road for frequency of consumption of cassava derivatives and bananas (makemba).

(Fig. 19). The progressive increase in the prevalence of goitre (see Fig. 16) followed that of the consumption of boiled, unsoaked cassava roots (*fuku*) and of cassava leaves (*mpondu*), and the decrease in the consumption of soaked cassava paste (*chickwangué*) and bananas (*makemba*).

## Discussion

The increase in the prevalence of goitre was associated on both roads with a similar increase in the serum levels of TSH and a decrease of  $T_4$ , as classically reported in endemic goitre (review by Delange and Ermans 1976). However, the relations between the prevalence of goitre and the degree of iodine defi-

ciency differed clearly from one road to the other. On the South-North road, iodine intake diminished progressively as the prevalence of goitre increased, in agreement with the classical inverse relationship apparent in most of the endemic goitre areas in the world (Stanbury et al. 1954; Podoba 1962; Schaefer 1974; Costa et al. 1975; review by Beckers and Delange 1980). In contrast, the West-East road was marked by a severe and uniform iodine deficiency. On this road, the increase in the prevalence of goitre was clearly related to a progressive increase in the urinary SCN concentrations.

In the Kungu zone, the prevalence of goitre is thus related either to a reduction in iodine intake in the presence of a high SCN intake (South-North road) or to an increasing SCN intake in the presence of a uniform iodine deficiency (West-East road). In consequence, our observations show that the nutritional factor responsible for the development of endemic goitre in this region is not the absolute iodine supply, but the balance between iodine and SCN in the diet. Along both roads, the urinary I/SCN ratio reached 3 for low goitre prevalences and was below 1 for high prevalences. Values of the same order are reported in chapter 3 and in an endemic goitre zone of Sicily (Delange et al. 1978).

Finally, our observations also show that the increase in the urinary SCN concentration is critically related to the consumption of cassava and the way in which this food is prepared. The highest concentrations are observed in those communities that eat mainly unsoaked roots and cassava leaves; these two methods of preparing cassava are very poor for eliminating the HCN contained in fresh cassava (Bourdoux et al. 1980a; Simons-Gérard et al. 1980; see also chapter 5).



## Chapter 8

# Serum Levels of Free Amino Acids in Mothers at Delivery, Newborns, and Adult Males

H.L. VIS, A. VUYE, AND P. HENNART

**Taux sériques des acides aminés libres chez la mère à l'accouchement, le nouveau-né et l'homme adulte — Résumé —** Le but principal de ce travail a été d'apprécier si l'état de malnutrition existant au Kivu et au Bas-Zaïre pourrait constituer un facteur limitant dans la conversion endogène du HCN libéré par le manioc en SCN. Ce processus nécessite en effet l'intervention d'acides aminés soufrés, notamment de méthionine.

Nous avons déterminé dans chacune des régions investiguées au Zaïre et à Bruxelles les taux des acides aminés libres du sérum dans quatre groupes de patients : des hommes adultes de 25 à 30 et de 30 à 35 ans, des femmes enceintes au moment de l'accouchement et des nouveau-nés (sang du cordon).

Dans les quatre groupes de patients, les taux sériques de méthionine sont pratiquement identiques dans les différentes régions investiguées (Tableaux 27–29, Fig. 20). Chez les adultes du Kivu, mais pas chez les mères ni chez les nouveau-nés, les taux de valine, leucine et isoleucine sont plus bas que chez les témoins, et le profil des acides

aminés libres est caractéristique d'un état de malnutrition sévère. Par contre, dans les quatre groupes de patients investigués en Ubangi, les taux sériques des acides aminés et en particulier de valine et de leucine sont pratiquement systématiquement plus élevés que chez les témoins.

Ce travail suggère fortement qu'au Kivu et au Bas-Zaïre, en dépit d'un état de malnutrition, l'apport en acides aminés soufrés est encore suffisant pour permettre la conversion endogène de HCN en SCN. Le profil en acides aminés libres au Kivu atteste, avec l'abaissement du taux sérique d'albumine, de la gravité de la malnutrition dans cette région. Le profil particulier des acides aminés libres en Ubangi n'est pas expliqué. Il pourrait résulter notamment de la consommation très importante de manioc ainsi que de l'insuffisance thyroïdienne endémique dans cette région.

As noted in chapters 2 and 3, malnutrition is endemic in Kivu and, to a lesser extent, in Bas Zaire. The question arises whether, in these two regions, malnutrition could interfere with the endogenous conversion of HCN into SCN, which requires sulfur amino acids such as methionine (Bourdoux et al. 1980a).

Therefore, we compared the serum levels of free amino acids in the four study areas in Zaire and in Brussels. These determinations also expand the base for assessment of the dietary supply of proteins given by serum albumin (Vis 1963).

## Patients and Methods

We obtained serum samples from clinically euthyroid patients in the four areas investigated in Zaire — Kinshasa (control), Bas Zaire, Kivu, and Ubangi — and in Brussels (control). For the different areas, serum pools from four patient groups were analyzed: adult males aged 25–30 and 30–35 years; pregnant women at delivery; and newborns, using cord serum samples derived from total blood, which is largely venous.

The serum samples collected in Zaire were frozen, then transferred from Africa to Brussels, where they were again stored at  $-20^{\circ}\text{C}$  for various intervals. Under such circumstances, the results obtained for some amino acids, in particular for glutamine, asparagine, serine, and proline, are known to be questionable. Consequently, the results for these amino acids were not considered closely, although most of them were consistent with those reported in the literature. The serum samples

were combined into pools, each containing at least 10 samples. Each pool was analyzed five times, and the interassay CV around the mean was less than 5%.

Chromatographic studies on ion exchange resin columns were made on a Technicon TSM apparatus.

## Results

In Kivu and Bas Zaire, the levels of serum methionine in each of the four age groups in-

vestigated were not lower than the ones found in the control areas (Tables 27–29, Fig. 20). In Kivu, the levels in mothers at delivery were slightly higher than in the controls.

The levels of serum free amino acids for the pools of the 25- to 30-year-old males were strikingly similar to the ones for 30- to 35-year-old males in the regions investigated (Table 27). In both groups of adult males in Kivu, but not in Bas Zaire, the serum levels of the branched free amino acids, valine, leucine, and isoleucine, were lower than in the controls. These differences were not found in Kivu, either for

Table 27. Serum levels of free amino acids in adult males aged 25–30 years and 30–35 years from three areas in Zaire and from Brussels. (Results are expressed in  $\mu\text{M/l}$  of serum.)

	Brussels		Bas Zaire		Kivu		Ubangi	
	25–30	30–35	25–30	30–35	25–30	30–35	25–30	30–35
Taurine	73	60	69	116	70	49	94	90
Threonine	204	201	203	223	130	156	326	298
(Serine)	270	264	321	341	196	240	327	268
Glycine	513	480	618	721	558	613	682	613
Alanine	718	697	766	859	611	711	888	873
Valine	357	341	278	293	212	270	404	394
Methionine	26	29	27	31	39	36	32	59
Isoleucine	112	111	109	117	75	88	105	129
Leucine	293	293	287	308	178	209	531	517
Tyrosine	105	100	79	90	58	63	150	143
Phenylalanine	156	148	138	152	109	115	214	221
Ornithine	187	167	148	167	116	132	229	205
Lysine	360	341	299	354	231	280	569	610
Histidine	150	141	129	139	108	127	155	148
Arginine	255	242	242	248	168	175	246	277
(Proline)	313	338	312	318	174	254	303	289

Table 28. Serum levels of free amino acids of the mothers at delivery from four areas in Zaire and from Brussels. (Results are expressed in  $\mu\text{M/l}$  of serum.)

	Brussels	Kinshasa	Bas Zaire	Kivu	Ubangi
Taurine	81	74	38	70	47
Threonine	220	270	309	359	395
(Serine)	52	308	382	359	477
Glycine	362	404	457	544	732
Alanine	476	699	738	870	1140
Valine	198	296	317	333	488
Methionine	31	29	28	75	48
Isoleucine	60	114	126	142	144
Leucine	208	491	547	606	799
Tyrosine	85	113	139	142	191
Phenylalanine	128	180	211	171	276
Ornithine	185	91	63	150	257
Lysine	216	375	554	590	682
Histidine	119	126	165	106	208
Arginine	44	397	559	336	392
(Proline)	92	275	283	305	434

Table 29. Free amino acids in cord serum (mixed venous and arterial blood) from four areas in Zaire and from Brussels compared with data from the literature. (Results are expressed in  $\mu\text{M/l}$  of serum.)

	Brussels	Kinshasa	Bas Zaire	Kivu	Ubangi	Reinhardt and Burkhalter	Pohlandt (1978)	
						(1980)	Vein	Artery
Taurine	86	79	71	71	89	175	91	85
Threonine	182	130	161	141	471	202	187	174
(Serine)	44	14	114	119	463	178	109	111
Glycine	293	189	286	331	723	281	195	195
Alanine	375	598	447	583	1274	453	330	262
Valine	177	234	182	200	664	214	191	158
Methionine	51	60	43	63	139	20	21	20
Isoleucine	43	54	62	66	204	61	42	43
Leucine	110	98	163	213	759	127	97	84
Tyrosine	47	60	48	62	241	59	48	43
Phenylalanine	67	104	96	109	367	93	53	48
Ornithine	109	90	71	89	162	83	60	53
Lysine	249	60	109	165	671	267	314	272
Histidine	59	68	75	75	169	83	96	88
Arginine			79	75	251	112	66	54
(Proline)	52	125	116	110	404	227	133	121

the mothers at delivery (Table 28), or for cord serum (Fig. 20).

In Ubangi, the levels of serum free amino acids, in particular valine and leucine, were nearly consistently higher in the four age groups than in the controls, especially in maternal serum at delivery (Table 28) and in cord serum (Fig. 20).

## Discussion

This study shows that, in Kivu and Bas Zaire, in spite of clinical and biochemical signs of protein calorie malnutrition, the serum levels of sulfur amino acids such as methionine are not lower than in the other study areas, including the control regions. Consequently, we cannot determine whether intake or utilization of sulfur amino acids is more or less important in one region than in another. However, this observation is consistent with the view that, in Kivu and Bas Zaire, the sulfur amino acids required for the endogenous conversion of HCN into SCN are still at least partly available. This conclusion ties in with the observation reported in chapter 2 of elevated levels of serum SCN in both areas.

The serum amino acid patterns found in adult males in Kivu, and in particular the low levels of serum branched free amino acids, valine, leucine, and isoleucine, are explained by the high prevalence of protein calorie malnu-

trition (Holt et al. 1963; Vis 1963), and are consistent with the low levels of serum albumin in the adult population reported in chapter 3. The reason that such differences are not observed in mothers at delivery or in cord serum in the same area, as was also observed from serum albumin (chapter 4), is not clear.

The observation that, in Bas Zaire, the serum amino acid pattern in adult males is unmodified, in spite of a slightly lower serum albumin found in a sample of adults of both sexes (chapter 3), further confirms that protein malnutrition is less severe in Bas Zaire than in Kivu.

Free amino acids in cord serum in Brussels are slightly lower than those reported by Reinhardt and Burkhalter (1980) for Ivory Coast but are generally in agreement with the findings reported by Pohlandt (1978) (Table 29) and by others (Ghadimi and Pecora 1964; Lindblad 1971; Hayashi et al. 1978; Senterre 1979; Boersma 1980). Hence, the levels found in Brussels can serve as a reference basis for the other regions (Fig. 20).

We have no accurate explanation for the peculiar amino acid pattern found in Ubangi. The pattern found in cord serum, together with the particularly low birth weight in this area (chapter 4), could indicate poor intrauterine nutrition. The possibility that a cassava-rich diet is involved cannot be excluded and could be supported by the consistently elevated levels of serum ornithine. The endemic hypothyroidism that is found in Ubangi could also be a con-



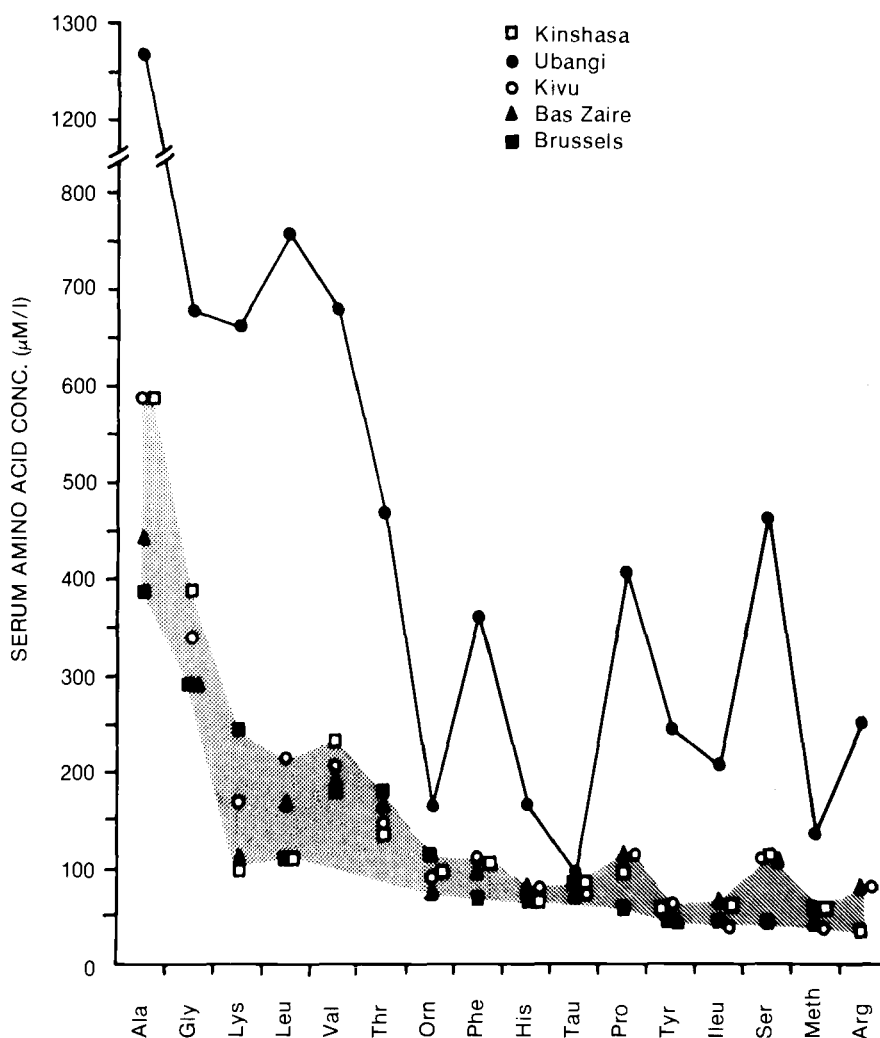


Fig. 20. Patterns of the free amino acids of cord serum. (Normal pattern shown by shading.)

tributing factor. The possible influence of hypothyroidism on amino acid metabolism and on the composition of the intra- and extracellular free amino pools requires further investigation.

In conclusion, the serum levels of sulfur amino acids are normal in adult males, mothers at delivery, and newborns in Kivu and Bas

Zaire, in spite of protein calorie malnutrition. This observation suggests that, in both areas, the endogenous conversion of HCN into SCN is not markedly impaired. The greater severity of malnutrition in Kivu than in Bas Zaire, shown by epidemiological data and by the levels of serum albumin, is further confirmed by the pattern of free amino acids.



## Chapter 9

# Experimental Study of Mechanisms Responsible for Mental Retardation Resulting from Cassava Ingestion

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**Étude expérimentale des mécanismes responsables du retard mental suite à la consommation de manioc — Résumé —** Le but du présent travail a été d'évaluer le rôle de la consommation de manioc par la mère durant la grossesse et la lactation sur le développement de lésions cérébrales irréversibles chez le nouveau-né et au début de la vie. Nous avons tenté d'apprécier expérimentalement chez le rat les rôles respectifs du HCN lui-même et de l'hypothyroïdie qu'il entraîne sur le développement de ces lésions.

Différents lots de rates gravides et allaitantes ont été soumises à des régimes carencés en iode avec ou sans suppléments en HCN, SCN et iodure (Tableaux 30 et 31). Les rats ont été sacrifiés à la naissance et à la fin de la période de lactation (16 jours) et le contenu des hémisphères cérébraux et du cervelet en acides nucléiques, protéines et li-

pides a été estimé afin de mettre en évidence d'éventuelles altérations du nombre, de la taille ou de la composition cellulaire. Les concentrations sériques de SCN et  $T_4$  ont également été déterminées.

L'administration aux rates d'un régime carencé en iode supplémenté en HCN provoque une hyperplasie thyroïdienne importante et une diminution de la  $T_4$  sérique aussi bien chez la mère que chez le raton, témoignant d'une insuffisance thyroïdienne. Ces effets sont plus importants que ceux provoqués par la carence iodée seule et sont corrigés par un supplément d'iodure. La surcharge en HCN avec ou sans supplément d'iodure est accompagnée d'une augmentation du SCN sérique (Tableau 32 et 35). Ces observations indiquent que l'hypothyroïdie induite par le HCN implique sa conversion préalable en SCN.

Le développement cérébral des rats hypothyroïdiens suite à l'administration de HCN aux rates carencées en iode est fondamentalement altéré. Les anomalies les plus frappantes sont observées au niveau du cervelet à l'âge de 16 jours où il existe une diminution très marquée du contenu en protéines, RNA et cholestérol traduisant un ralentissement de la croissance cellulaire. Ces anomalies ne sont plus observées lorsque la même surcharge en HCN est administrée en présence d'un apport iodé normal (Tableau 39).

Quel que soit le régime administré aux différents groupes expérimentaux et le degré d'hypothyroïdie induit, le contenu du cerveau en RNA, utilisé comme index du processus de croissance cellulaire, demeure remarquablement constant aussi longtemps que la  $T_4$  sérique reste supérieure à un seuil critique de  $1 \mu\text{g/dl}$ . Au-dessous de ce seuil, le RNA s'effondre brusquement (Fig. 21).

Toutefois, l'administration de très grandes quantités de SCN est également susceptible d'abaisser le RNA cérébral même lorsque l'administration concomitante d'iodure prévient le développement d'une hypothyroïdie.

En conclusion, l'effet du HCN sur le développement cérébral du jeune rat carencé en iode semble résulter essentiellement de l'insuffisance thyroïdienne qu'il induit après sa conversion en SCN. Le HCN ne semble avoir que peu ou pas d'action toxique directe sur le cerveau. Il n'est cependant pas exclu qu'à hautes doses, le SCN puisse exercer par lui-même une action toxique.

Ces observations soulignent le risque d'altérations irréversibles du système nerveux central chez le jeune enfant suite à l'ingestion de manioc par la femme enceinte, en particulier en présence d'une carence iodée.

The major complication of endemic goitre is endemic cretinism (review by Pharoah et al. 1980), which is defined by the association of irreversible mental retardation and signs of hypothyroidism (myxedematous cretinism) or of impairment of the central nervous system (neurologic cretinism) (McCarrison 1908; Dumont et al. 1969; Delange et al. 1972c; Querido et al. 1974). In addition, clearcut mental retardation may also be present in endemic goitre regions in patients who do not present the other signs of cretinism and, in particular, no evidence of thyroid insufficiency (Ramirez et al. 1969; Ibbertson et al. 1971; Greene 1973; Fierro-Benitez et al. 1974a, 1974b; Lagasse et al. 1980c).

The mechanisms responsible for mental retardation in endemic goitre are only partly understood. In myxedematous cretinism, the degree of growth and mental retardation in adult cretins (Bastenie et al. 1962; Dumont et al. 1963; Delange et al. 1972b) and some radiological findings (Delange 1974) suggest that hypothyroidism occurring during fetal or early postnatal life may be the responsible factor. The demonstration of biochemical features of severe hypothyroidism in a large number of newborns and young children in the endemic area of Ubangi (Delange et al. 1976, 1980a; Thilly et al. 1978, 1980a; see also chapter 3, 4, and 6) supports this hypothesis. The observed congenital hypothyroidism is the joint result of very severe iodine deficiency in the mother during pregnancy (Thilly et al. 1978) aggravated by SCN overload resulting from cassava consumption (Delange et al. 1980a).

One can ask whether cassava ingestion may be directly involved in the development of mental retardation in endemic goitre, independent of the hypothyroidism that it causes. The hydrolysis of linamarin liberates HCN (Bourdoux et al. 1980a). Experimentally, chronic HCN intoxication, in turn, produces irreversible damage to the central nervous system (Hurst 1940; Smith et al. 1963; Brierley et al. 1976).

The purpose of the present study was to evaluate the role of cassava consumption by the mother during pregnancy and lactation on the development of irreversible cerebral lesions in the newborn and during the first weeks of life. Specifically, we attempted to set up an experimental model that assessed the respective roles of HCN itself and of the resultant hypothyroidism in the development of these lesions. This experimental study of en-

demic cretinism was performed in rats, whose cerebral development is essentially postnatal (Dobbing 1974).

The consequences of neonatal hypothyroidism on the central nervous system in this species have been the subject of a number of studies (review papers by Balazs et al. 1971; Balazs 1972, 1977; Brasel and Boyd 1975; Bass et al. 1977; Ford and Cramer 1977; Hamburgh et al. 1977; Rosman and Malone 1977; Sokoloff 1977; Ruiz-Marcos et al. 1979; Morreale de Escobar and Escobar del Rey 1980). The main conclusions are:

- Thyroid insufficiency affects cell acquisition, migration, and differentiation.
- All regions of the brain are involved although to different extents as a function of developmental chronology.
- Neonatal hypothyroidism in the rat engenders permanent sequelae if not corrected before a critical age starting at day 14.
- At the end of the period of cerebral maturation, i.e., 30 days, the brain is reduced in size and the neurones are markedly hypoplastic.
- Cerebral DNA concentration is increased and cellular RNA, protein, and lipid contents are reduced.

The effect of HCN on the adult rat brain has been well documented. HCN inhibition of cytochrome oxidase (Keilin 1930; Pudek and Bragg 1974) induces changes in the intracellular redox reactions (Albaum et al. 1946) with a shift from aerobic to anaerobic pathways (Isom et al. 1975). This results in lactate accumulation, decreased ATP concentration, and increased ADP concentration leading to degeneration of nervous tissues (Smith et al. 1963). Bass (1968) has shown that glial cells are preferentially affected with myelin destruction as a consequence.

To our knowledge, however, the direct action of HCN on the brain in the newborn and suckling rat has not been documented. Our results suggest that HCN itself has little or no toxic effect on central nervous system development in the young rat. The cerebral lesions caused by HCN in the presence of iodine deficiency are the result of induced hypothyroidism.

## Materials and Methods

We compared the effects of different diets fed to pregnant and lactating rats on thyroid

function and cerebral development in their progeny at birth and at the end of lactation (16 days). We used diets (Remington) deficient in iodine supplemented with HCN or SCN and with or without added iodide. A series of measurements were made on the brains of rats at birth and at 16 days of life. Brain weight and DNA, RNA, and protein contents were determined as an indication of cell number, density, and size. Cholesterol content was taken as an aspecific index of lipid accumulation in cellular membranes. In certain instances, cerebral cerebrosides were measured, allowing estimation of the degree of myelination of axones and dendrites. In addition, thyroid weight and serum  $T_4$  and SCN concentrations were determined in the mothers and pups.

## Animals

Wistar rats weighing 195–270 g were obtained on the 1st day of pregnancy (Proefdieren Centrum, KUL, Leuven) and grouped according to diet. The diets were fed from the 1st day until the end of lactation, i.e., 16th day of life of the progeny. Half of the mothers and progeny were sacrificed (decapitation) at the birth of the young, the rest at 16 days. Brains of the young rats were immediately removed; the cerebral hemispheres and cerebellum were separated, weighed, and stored at  $-20^{\circ}\text{C}$ .

## Methods

**Homogenization:** Tissue samples were homogenized in cold perchloric acid (PCA) 0.4 N (20% weight : volume). After centrifugation at 3000 rpm for 20 min at  $4^{\circ}\text{C}$ , the pellet was washed and resuspended in 0.2 N PCA. The supernatant was discarded.

**Lipid extraction and purification:** The residue was treated successively with cold sodium acetate-saturated ethanol, chloroform-methanol (2 : 1, v/v) twice, and ether. The latter extractions were carried out at room temperature. Finally, the residue was again treated with acetate-saturated alcohol in the cold.

The lipids were purified (Folch et al. 1957): the lipid extracts were dried under nitrogen and then solubilized in chloroform-methanol-water (16 : 8 : 1), 20 volumes/g of tissue (wet weight). A two-phase separation was then performed by the addition of 0.2 volumes NaCl (0.9 g%). The two phases were separated and dried under nitrogen. The upper

phase contained the gangliosides and the lower phase contained the other lipids.

**Proteins and nucleic acids:** Proteins and nucleic acids were purified and measured according to the method described by Schmidt-Thannhauser (1945) as modified by Wannemacher et al. (1965) and by Balazs and Patel (personal communication).

**RNA extraction:** The residue was solubilized in 0.3 N NaOH (12 volumes/g of tissue wet weight) for 1 hour at  $37^{\circ}\text{C}$  (Fleck and Munro 1962). A portion of the hydrolysate was reserved for protein determination. To the rest, 3 N PCA was added so that the final suspension was 0.2 N with respect to PCA. Under these conditions, DNA and proteins precipitated but RNA remained in solution. The suspension was centrifuged at  $4^{\circ}\text{C}$ , the pellet was washed with cold 0.2 N PCA and recentrifuged. The resulting supernatants were combined and the RNA content was determined.

**DNA extraction:** DNA was extracted in 1 N PCA at  $70^{\circ}\text{C}$  for 20 min. After centrifugation, the pellet was washed with 0.2 N PCA. The combined supernatants were used to quantify DNA.

## Analytical methods

**Cholesterol determination:** The assay was performed on an aliquot of the lower lipid phase taken up in chloroform. Cholesterol was measured according to the technique of Abell et al. (1952) using a mixture of acetic anhydride- $\text{H}_2\text{SO}_4$ -acetic acid (modified Liebermann-Burchard reagent). Optical density was read at 620 nm using samples of 0.5 ml containing about 1–2 mg/ml cholesterol. Standard pure cholesterol was obtained from Merck.

**Cerebroside determination:** The method used was a modification (Hess and Lewin 1965) of that described by Svennerholm (1956). Aliquots of the lower phase were analyzed as galactose using an orcinol- $\text{H}_2\text{SO}_4$  mixture. According to the modification suggested by Balazs et al. (1971), the colour was extracted into chloroform : methanol (1 : 1) and read at 440 nm. The samples contained 0–10  $\mu\text{g}$  galactose and the standard was D(+)-galactose (Merck).

**Protein determination:** Proteins were quantified according to Lowry et al. (1951) permitting measurement of 25–500  $\mu\text{g}$  protein/ml of sam-

ple. The protein was reacted with an alkaline copper solution and then with phosphomolybdotungstate (Folin–Ciocalteu reagent). The coloured complex was read at 500 nm against a crystalline bovine albumin standard (Calbiochem).

**RNA determination:** Aliquots of the RNA fraction were appropriately diluted in 0.1 N PCA and their absorbance was measured at 260 and at 233 and 280 nm to evaluate the purity of the extract and the absence of contaminating protein. The concentration of RNA-P was calculated on the basis that purified RNA extracted from brain at a concentration of 1  $\mu\text{g}$  atom RNA-P/ml had an extinction coefficient of 10.5 at a wavelength of 260 nm.

**DNA determination:** DNA was measured by the method of Burton (1956) which allows the determination of 0.02–0.25  $\mu\text{g}$  atom DNA-P/ml. The method was based on the reaction between deoxyribose and diphenylamine. Sensitivity was enhanced in the presence of perchloric acid and acetaldehyde. Colour development required incubation at 30°C for 16–20 hours. The samples were read at 600 nm against calf thymus DNA (Merck). Samples were diluted in 0.5 N PCA.

**Serum concentrations of SCN and  $T_4$ :** The methods used to determine SCN and  $T_4$  are described in chapter 1.

## Experimental protocols

The investigations were carried out in two stages. In the first (experiment 1), we attempted to demonstrate as many effects as possible of HCN and SCN on thyroid function and cerebral development in the young rat. We compared the effects of HCN and SCN in the presence or absence of iodide using controls that were not rendered iodine deficient. The animals in the five experimental groups in experiment 1 (Table 30) were administered an iodine-deficient diet (Remington diet, LID) and supplements. Groups 4 and 5 received SCN that corresponded on a molar basis to the amount of HCN given to groups 2 and 3. In groups 3 and 5, the antithyroid effects of HCN and SCN were compensated for by iodide supplementation. The investigation involved a total of 16 mother rats and 138 pups with the number of pups suckling one mother not being limited.

Table 30. Experiment 1: Supplements (expressed per 100 g of rat body weight per day) added to basal iodine-deficient diet (LID).

Group	Diet	Supplement		
		I <sup>-</sup> ( $\mu\text{g}$ )	CN <sup>-</sup> (mg)	SCN <sup>-</sup> (mg)
1	Control	10		
2	CN		8	
3	CN + I	10	8	
4	SCN			17.8
5	SCN + I	10		17.8

The following variables were measured:

- Body weight and weight of the thyroid gland.
- Serum SCN and  $T_4$  concentrations.
- Brain content of proteins, nucleic acids, cholesterol, and cerebroside.

As experiment 1 demonstrated that certain HCN and SCN effects could be due to thyroid insufficiency, we undertook a second experiment in which the effects of HCN were compared to the effects of iodine deficiency alone. Since SCN effects were not tested further, the number of animals per group could be increased and their diets could be more accurately controlled. In addition, each mother nursed an identical number of pups, thus reducing as much as possible the variability in body weight between animals.

All the animals in experiment 2 received the same iodine-deficient diet as in experiment 1 with iodide and HCN supplements (Table 31). The investigation involved a total of 24 mothers and 183 pups and the following variables were measured:

- Body weight and weight of the thyroid gland and serum SCN and  $T_4$  concentrations in the mothers and pups.
- Brain weight and water, protein, nucleic acid, and cholesterol contents of the brain in the pups at birth and at 16 days.

All methods were as described above except that the homogenization of brain tissue was performed in 10 volumes of distilled water at 4°C immediately after sacrifice. An aliquot of the homogenate (usually 1 ml) was dried at 110°C for 48 hours to determine brain water content.

Table 31. Experiment 2: Supplements (expressed per 100 g body weight per day) added to basal iodine-deficient diet (LID).

Group	Diet	Supplement	
		I <sup>-</sup> (μg)	CN <sup>-</sup> (mg)
A	Control	10	
B	I deficient		
C	CN		8
D	CN + I	10	8

## Results

### Experiment 1

No differences in body weight were observed between the experimental groups for either the mothers or the pups.

*Thyroid and serum:* Marked thyroid hyperplasia was observed in mothers and their progeny following administration of CN and particularly of SCN (Table 32). An increase in serum SCN and a decrease in serum T<sub>4</sub> accompanied the thyroid hyperplasia. Iodine supplementation prevented these changes with the exception of increased serum SCN.

*Cerebral hemispheres and cerebellum:* No significant differences in brain weight were observed between experimental groups at day 1 or day 16. The results for biochemical composition (proteins, RNA, and cholesterol) of cerebral hemispheres (Table 33) are expressed per micromole of DNA-P to account for cellular growth.

The biochemical composition at birth of the cerebral hemispheres of rats whose mothers were subjected to an HCN-supplemented diet did not differ from that of the controls. In contrast, SCN supplementation (group 4) resulted in decreased cerebral protein, RNA, and cholesterol. These modifications were not corrected by iodine supplementation (group 5).

The cerebral protein, RNA, and cholesterol contents were greater at day 16 than at day 1 in keeping with the growth of the pups (Table 33). As at day 1, the results in groups 2 and 3 were comparable to those in the control group except for cholesterol content, which was moderately decreased in group 3. The decreased values in group 4 were again evident, but partially corrected by iodine supplementation (group 5). The decrease was particularly marked for RNA and cerebroside in group 4.

In the cerebellum of the pups at day 16 (Table 34), the group fed an HCN-supplemented diet (group 2) showed increased protein, RNA, and cerebroside contents. These increases were corrected by iodine supplementation (group 3) except for cerebroside. The results in group 4 (SCN) were similar to those in the cerebral hemispheres on day 1, i.e., decreased protein and RNA content not reversed by iodine supplementation. However, cholesterol and cerebroside content were not significantly modified.

### Experiment 2

No significant differences in body weight were detected among the groups of mothers or pups.

Table 32. Thyroid weight and concentrations of serum SCN and T<sub>4</sub> in mother rats (M) and progeny (P) at 16 days. (Mean ± SD. Numbers of pups in each group in parentheses.)

Group	Diet	Thyroid weight (mg/100 g body weight)		Serum concentration			
				SCN (mg/dl)		T <sub>4</sub> (μg/dl)	
		M	P	M	P	M	P
1	Control	8.6 (2)	19.7 (1)	0.22 (2)	0.46 (2)	4.9 (2)	7.0 (2)
2	CN	23 (2)	139 (2)	1.11±0.12 (4)	0.57±0.14 (3)	0.8±0.3 (4)	<0.5 (3)
3	CN + I	7.8 (2)	21.7 (1)	1.43 (2)	0.61 (2)	4.8 (2)	4.8 (2)
4	SCN	59 (2)	-	>4 (2)	0.77 (2)	<0.5 (2)	<0.5 (2)
5	SCN + I	9.3 (2)	20 (2)	>4 (2)	0.97 (2)	2.4 (2)	5.3 (2)

Table 33. Biochemical composition of cerebral hemispheres of pups at birth and at day 16. (Results are expressed as means per  $\mu\text{mole DNA-P} \pm \text{SD}$ .<sup>a</sup> Numbers of pups are shown in parentheses.)

Group	Diet	Proteins (mg)	RNA ( $\mu\text{mole P}$ )	Cholesterol (mg)	Cerebrosides ( $\mu\text{g galactose}$ )
<b>At birth</b>					
1	Control	$7.51 \pm 0.45$ (11)	$1.32 \pm 0.06$ (11)	$0.42 \pm 0.02$ (8)	—
2	CN	$7.58 \pm 0.57\text{NS}$ (12)	$1.31 \pm 0.08\text{NS}$ (12)	—	—
3	CN + I	$8.00 \pm 0.49\text{NS}$ (10)	$1.41 \pm 0.09\text{NS}$ (10)	$0.43 \pm 0.03\text{NS}$ (10)	—
4	SCN	$6.29 \pm 0.39^{***}$ (9)	$1.05 \pm 0.04^{***}$ (10)	$0.35 \pm 0.01^{***}$ (6)	—
5	SCN + I	$6.26 \pm 0.32^{***}$ (12)	$1.07 \pm 0.03^{***}$ (12)	$0.34 \pm 0.01^{***}$ (12)	—
<b>At day 16</b>					
1	Control	$29.6 \pm 2.1$ (12)	$3.51 \pm 0.26$ (12)	$3.81 \pm 0.19$ (6)	$54 \pm 7$ (7)
2	CN	$28.9 \pm 2.3\text{NS}$ (5)	$3.28 \pm 0.28\text{NS}$ (5)	—	$59 \pm 7\text{NS}$ (5)
3	CN + I	$29.2 \pm 1.6\text{NS}$ (8)	$3.34 \pm 0.14\text{NS}$ (8)	$3.48 \pm 0.19^*$ (8)	$53 \pm 7\text{NS}$ (8)
4	SCN	$25.7 \pm 0.6^{***}$ (5)	$2.76 \pm 0.05^{***}$ (5)	$3.05 \pm 0.09^{**}$ (5)	$37 \pm 6^{**}$ (5)
5	SCN + I	$29.4 \pm 1.7\text{NS}$ (9)	$3.28 \pm 0.17\text{NS}$ (11)	$3.44 \pm 0.30^*$ (8)	$41 \pm 6^{**}$ (6)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Table 34. Biochemical composition of cerebellum at day 16. (Results are expressed as means per  $\mu\text{mole DNA-P} \pm \text{SD}$ .<sup>a</sup> Numbers of pups are given in parentheses.)

Group	Diet	Proteins (mg)	RNA ( $\mu\text{mole P}$ )	Cholesterol (mg)	Cerebrosides ( $\mu\text{g galactose}$ )
1	Control	$4.11 \pm 0.20$ (10)	$0.57 \pm 0.03$ (9)	$0.32 \pm 0.03$ (10)	$12.9 \pm 2.0$ (5)
2	CN	$4.41 \pm 0.16^{**}$ (6)	$0.63 \pm 0.01^{***}$ (6)	—	$16.7 \pm 1.8^*$ (5)
3	CN + I	$4.17 \pm 0.12\text{NS}$ (7)	$0.58 \pm 0.03\text{NS}$ (8)	$0.35 \pm 0.01^*$ (8)	$17.2 \pm 1.9^{**}$ (7)
4	SCN	$3.71 \pm 0.08^{***}$ (5)	$0.52 \pm 0.01^{**}$ (5)	$0.30 \pm 0.02\text{NS}$ (5)	$13.7 \pm 1.1\text{NS}$ (4)
5	SCN + I	$3.78 \pm 0.15^{***}$ (12)	$0.55 \pm 0.01^*$ (12)	$0.31 \pm 0.02\text{NS}$ (12)	$12.8 \pm 1.3\text{NS}$ (6)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Mortality and anomalies:** In the HCN-supplemented group (C), the litter size was highly variable (1–12 pups) whereas there were always about 11 pups in the other groups. Among the 40 pups of group C, two presented striking malformations at birth, one had hind-leg paralysis and the other dystrophy of a front limb. In group D (HCN and iodide), although no anomalies were detected at birth, 2 of the 17 pups were highly abnormal at 16 days: they

were underdeveloped (weight about 15 g) and presented seizures. At autopsy, their brains were edematous and mushy in consistency. However, these animals did not have goitres and their serum  $T_4$  concentration was normal.

**Thyroid and serum:** The results for thyroid weight and serum SCN and  $T_4$  concentrations (Table 35) complemented those obtained in experiment 1. They confirmed the presence of

Table 35. Thyroid weight and serum SCN and T<sub>4</sub> concentrations in mother rats (M) and progeny (P) at birth and at day 16. (Mean  $\pm$  SD.<sup>a</sup> Numbers of pups in each group are given in parentheses.)

Group	Diet	Thyroid weight (mg/100 g body weight)		Serum concentration			
				SCN (mg/dl)		T <sub>4</sub> (μg/dl)	
		M	P	M	P	M	P
<b>At birth</b>							
A	Control	6.5±1.0 (4)	14±4 (10)	0.76±0.15 (4)	0.48±0.12 (3)	3.8±1.2 (4)	0.7±0.1 (5)
B	I deficient	9.3±0.4** (4)	40±12*** (13)	0.38±0.03** (4)	0.27±0.05* (4)	1.3±0.4** (4)	0.6±0.3NS (4)
C	CN	17±6* (4)	73±17*** (4)	1.04±0.16* (4)	1.35±0.92NS (3)	0.7±0.2** (4)	<0.5 (5)
D	CN + I	6.3±0.8NS (3)	12±5NS (6)	0.99±0.12NS (3)	1.19±0.41NS (4)	3.7±0.5NS (3)	0.6±0.1NS (4)
<b>At day 16</b>							
A	Control	6.1±1.6 (4)	11±3 (10)	0.40±0.05 (4)	0.43±0.06 (4)	4.7±1.8 (4)	7.7±0.6 (4)
B	I deficient	13±8NS (3)	63±31*** (6)	0.57±0.07** (4)	0.52±0.02NS (3)	1.2±0.1* (3)	1.5±0.9*** (9)
C	CN	18±11NS (3)	111±55*** (5)	1.21±0.11*** (3)	0.82±0.47NS (3)	0.7±0.1* (3)	0.5±0.1*** (8)
D	CN + I	5.8±0.3NS (3)	12±2NS (6)	1.23±0.41** (3)	0.75±0.03*** (3)	4.5±1.1NS (3)	7.6±0.2NS (3)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

thyroid hyperplasia in mothers and their progeny subjected to iodine deficiency (groups B and C). They demonstrated the goitrogenic effect of HCN administration, the manifestations of thyroid insufficiency being, in general, more marked in the HCN-treated group (C) than in the iodine-deficient group (B). The correlation between the degree of hypothyroidism in mothers and in pups was clearly apparent.

*Cerebral hemisphere and cerebellum:* Wet weight and water content of cerebral hemisphere at day 1 (Table 36) showed that, with the exception of a slightly increased weight in iodine-deficient and HCN-treated groups (B and C), the experimental groups did not differ significantly. For the cerebral hemispheres at day 16 (Table 36), wet weight and water content were significantly increased in the HCN groups (C and D). In the cerebellum, only wet weight was increased (Table 37).

Biochemical composition of the cerebral hemispheres of pups at birth (Table 38) showed no significant differences between groups, with the exception of a slightly higher RNA content in the HCN-treated group (C).

Table 36. Wet weight and water content of cerebral hemispheres at birth and at day 16. (Mean  $\pm$  SD.<sup>a</sup> Numbers of pups in each group are given in parentheses.)

Group	Diet	Wet weight (g)	% H <sub>2</sub> O
<b>At birth</b>			
A	Control	0.1675 $\pm$ 0.0066 (20)	87.4 $\pm$ 0.3 (8)
B	I deficient	0.1751 $\pm$ 0.0077** (19)	87.2 $\pm$ 0.2NS (7)
C	CN	0.1753 $\pm$ 0.0117* (16)	87.4 $\pm$ 0.1NS (6)
D	CN + I	0.1679 $\pm$ 0.0128NS (16)	87.4 $\pm$ 0.2NS (5)
<b>At day 16</b>			
A	Control	0.9231 $\pm$ 0.0516 (12)	83.7 $\pm$ 0.2 (9)
B	I deficient	0.9346 $\pm$ 0.0582NS (12)	83.3 $\pm$ 0.6NS (9)
C	CN	0.9599 $\pm$ 0.0231* (12)	84.3 $\pm$ 0.5** (9)
D	CN + I	0.9581 $\pm$ 0.0350NS (11)	83.9 $\pm$ 0.2* (9)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*\*, P < 0.01.



Table 37. Wet weight and water content of cerebellum at day 16. (Mean  $\pm$  SD.<sup>a</sup> Numbers of pups are given in parentheses.)

Group	Diet	Wet weight (g)	% H <sub>2</sub> O
A	Control	0.1350 $\pm$ 0.0077 (11)	81.8 $\pm$ 0.8 (6)
B	I deficient	0.1595 $\pm$ 0.0054*** (10)	81.9 $\pm$ 0.3NS (6)
C	CN	0.1449 $\pm$ 0.0086** (12)	82.5 $\pm$ 0.5NS (6)
D	CN + I	0.1542 $\pm$ 0.0183** (11)	82.4 $\pm$ 0.2NS (5)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*\*, P < 0.01; \*\*\*, P < 0.001.

In the iodine-deficient group (B) at day 16, some cellular hypertrophy was evident. RNA content was significantly reduced in group C. This anomaly had been corrected by iodine supplementation (group D), and no significant changes in cholesterol content were observed.

The biochemical composition of cerebellum at day 16 (Table 39) showed again that the modifications observed in iodine-deficient and HCN-treated groups (B and C) were in opposite directions. In group B, protein and RNA content was increased and cholesterol unchanged whereas in group C all parameters were decreased. Iodine supplementation (group D) normalized the results.

Table 38. Biochemical composition of cerebral hemispheres at birth and at day 16. (Results are expressed as means per  $\mu$ mole DNA-P  $\pm$  SD.<sup>a</sup> Numbers of pups are given in parentheses.)

Group	Diet	Proteins (mg)	RNA ( $\mu$ mole P)	Cholesterol (mg)
<b>At birth</b>				
A	Control	6.77 $\pm$ 0.32 (8)	1.04 $\pm$ 0.02 (8)	0.35 $\pm$ 0.01 (4)
B	I deficient	6.74 $\pm$ 0.34NS (8)	1.07 $\pm$ 0.04NS (8)	0.36 $\pm$ 0.02NS (4)
C	CN	7.01 $\pm$ 0.55NS (6)	1.12 $\pm$ 0.07** (6)	0.36 $\pm$ 0.02NS (4)
D	CN + I	6.53 $\pm$ 0.24NS (5)	1.02 $\pm$ 0.03NS (5)	0.35 $\pm$ 0.01NS (4)
<b>At day 16</b>				
A	Control	30.7 $\pm$ 1.7 (10)	3.23 $\pm$ 0.16 (10)	3.22 $\pm$ 0.19 (9)
B	I deficient	34.3 $\pm$ 2.8** (10)	3.67 $\pm$ 0.23*** (10)	3.42 $\pm$ 0.33NS (10)
C	CN	30.0 $\pm$ 1.4NS (9)	2.98 $\pm$ 0.16** (10)	3.17 $\pm$ 0.22NS (10)
D	CN + I	28.7 $\pm$ 1.0NS (9)	3.10 $\pm$ 0.09NS (9)	3.12 $\pm$ 0.11NS (9)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*\*, P < 0.01; \*\*\*, P < 0.001.

Table 39. Biochemical composition of cerebellum at day 16. (Results are expressed as means per  $\mu$ mole DNA-P  $\pm$  SD.<sup>a</sup> Numbers of pups are given in parentheses.)

Group	Diet	Proteins (mg)	RNA ( $\mu$ mole P)	Cholesterol (mg)
A	Control	4.00 $\pm$ 0.28 (6)	0.55 $\pm$ 0.04 (6)	0.31 $\pm$ 0.02 (6)
B	I deficient	4.48 $\pm$ 0.19** (6)	0.60 $\pm$ 0.01* (6)	0.32 $\pm$ 0.02NS (6)
C	CN	3.45 $\pm$ 0.18*** (6)	0.47 $\pm$ 0.02** (6)	0.27 $\pm$ 0.02** (6)
D	CN + I	4.03 $\pm$ 0.30NS (5)	0.55 $\pm$ 0.04NS (5)	0.31 $\pm$ 0.02NS (5)

<sup>a</sup>Levels of significance refer to the comparison with the results from the control group: NS, nonsignificant; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

*Relation between serum T<sub>4</sub> and cerebral RNA:* The diets administered to pregnant and nursing rats in the two experiments induced variable degrees of thyroid hyperplasia and variable decreases in serum T<sub>4</sub>. It therefore seemed of interest to look for a possible relationship between serum T<sub>4</sub> concentrations as an indication of thyroid function and RNA content of cerebral hemispheres and cerebellum as an indication of cerebral cellular growth.

Both for the cerebral hemispheres and the cerebellum at 16 days, i.e., near the end of the period of cerebral growth, RNA concentration was remarkably constant from one group to another, whatever the diet, for T<sub>4</sub> concentrations greater than 1 µg/dl (Fig. 21). In contrast, when serum T<sub>4</sub> was lower than this critical threshold, independent of the diet responsible, RNA concentration decreased sharply. This indicated a drastic slowing of cerebral growth.

## Discussion

Administration of an iodine-deficient diet alone or such a diet supplemented with HCN to pregnant and nursing rats induces marked thyroid hyperplasia and decreases serum T<sub>4</sub> concentrations in both mothers and offspring, indicating a state of thyroid insufficiency (Studer et al. 1974). Thyroid hyperplasia is more striking in the pups than in their mothers and much more important in the pups at the end of lactation (day 16) than at birth (Tables 32 and 35). The effect of iodine deficiency plus HCN on thyroid weight and serum T<sub>4</sub> levels is greater than that of iodine deficiency alone. These changes are not apparent when HCN overload is associated with normal iodine supply. HCN overload, with or without iodine supplementation, is accompanied by a highly significant increase in serum SCN concentration.

These observations, in particular the aggravating effect of lactation (Table 35), are identical to those reported previously when the goitrogenic agent administered to the mother was SCN (Delange et al. 1980b). The results strongly suggest that the goitrogenic action of HCN involves its conversion into SCN, as shown previously in adult rats (Kreutler et al. 1978; Ermans et al. 1980b).

HCN-induced hypothyroidism in experiment 2 is accompanied by a striking decrease in the protein, RNA, and cholesterol contents of the cerebellum at day 16 (Table 39), indicat-

ing a slowing of cellular growth. The principal effect of HCN in the cerebral hemispheres is to lower RNA concentration (Table 38). These findings confirm that the cerebellum is more sensitive to the effects of hypothyroidism than the cerebral cortex in the newborn rat at birth and during the critical period of cerebral development (review in Grave 1977). However, these alterations in brain composition are prevented when iodine supply is normal.

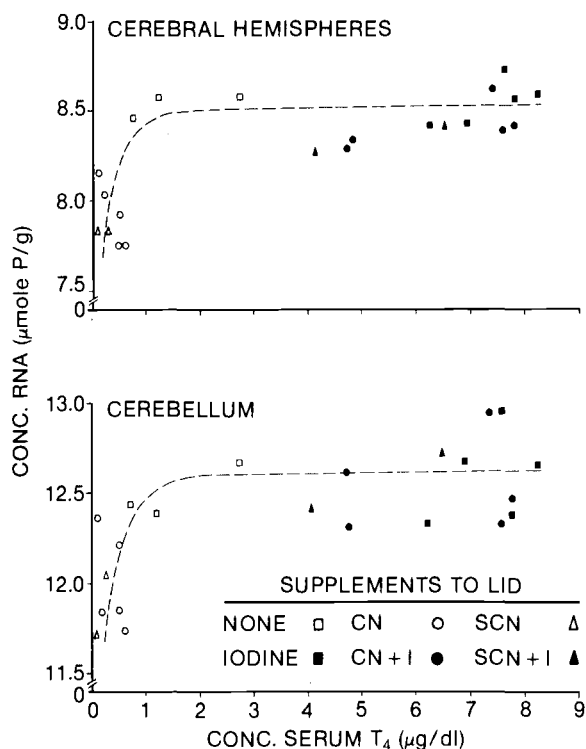
In experiment 1, the protein and RNA contents at 16 days of the cerebellum of pups of HCN-supplemented mothers are higher than in the controls (Table 34), contrasting with lower values found in experiment 2 (Table 39), which was more strictly monitored. This discrepancy could be at least partly explained if HCN-induced hypothyroidism was delayed in the preliminary experiment; Gourdon et al. (1973) have shown that, in the early stage of hypothyroidism, cellular multiplication is more impaired than cellular growth in the cerebellum of young rats.

For all the experimental groups studied, whatever the diet, brain RNA, used as an index of the process of cellular growth, is remarkably constant both in the cerebral hemispheres and in the cerebellum in spite of variations of serum T<sub>4</sub> concentrations ranging from a normal of about 7 µg/dl to as low as 1 µg/dl in rats at 16 days. However, when serum T<sub>4</sub> falls below this critical threshold, RNA content drops sharply.

Taken together these data confirm that severe hypothyroidism during the neonatal period in the rat, whatever its cause, produces alterations in the maturation of the central nervous system (Grave 1977). They strongly suggest that HCN does not affect these processes directly but acts indirectly by producing hypothyroidism after being converted to SCN.

It is possible, however, that the increase in weight and water content of the cerebral hemispheres at day 16 in animals receiving HCN with or without iodide supplementation (Table 36) could correspond to cytotoxic edema (Klatzo 1967) caused directly by HCN independent of an effect on thyroid function. HCN intoxication could also account for the neurological signs present in 4 of the 90 pups in experiment 2. These signs bear some resemblance to those in human adults with tropical neuropathy secondary to chronic HCN intoxication (Osuntokun 1973).

One unexpected aspect of this study is the observation (Table 33) that SCN overload,



**Fig. 21.** Relationship between serum  $T_4$  concentration and RNA content for cerebral hemispheres and for cerebellum in pups at day 16 in all experimental groups. (Each point represents mean for a group of 3–7 animals.)

even in the presence of normal iodine supply, provokes an important decrease in protein, RNA, and lipid content of the brain, an effect already evident at birth. These were the most dramatic changes observed during the study. We must point out, however, that the supplement of SCN that produced these effects (17.8 mg/day) is 10–20 times higher than the maximum amounts of SCN supplied to adult rats fed ad libitum exclusively with cassava (1–2 mg SCN/day) (Ermans et al. 1980b). Thus, it is possible that SCN in pharmacological doses, independent of its action on thyroid function, has a direct cytotoxic effect on brain development. Such an action has already been reported in spinal cord (Goto and Esplin 1961) and mammary gland (Pyska 1977).

The increase of protein and RNA in the cerebral cortex and cerebellum at 16 days in the iodine-deficient groups is compatible with the hypothesis that iodine deficiency early in the process of brain development might slow cellular multiplication. This hypothesis, in turn, is compatible with a direct action of iodine on cerebral maturation, independent of its action on thyroid function, as proposed by Hetzel and Hay (1979). Nevertheless, it is difficult to

specify the roles of iodine deficiency as such and thyroid insufficiency on the basis of our experiments (Table 35).

In conclusion, this study shows that the administration of HCN to pregnant rats in the presence of iodine deficiency hinders the cerebral development of their newborn and suckling offspring. The effect of HCN on the cerebral development is mediated by the thyroid insufficiency resulting from the endogenous conversion of HCN into SCN. HCN appears to have little or no direct toxic effect on the brain during this period. In contrast, huge amounts of SCN administered during pregnancy and lactation may cause cerebral anomalies even in the presence of normal iodine supply and thus in the absence of hypothyroidism.

Extrapolated to humans, the results of these investigations underline the important risk of irreversible alterations in cerebral development in the young child and thus the risk of endemic cretinism associated with cassava ingestion by pregnant mothers and nursing infants, particularly in the presence of iodine deficiency.



## Chapter 10

# Summary and General Conclusions

F. DELANGE, P. BOURDOUX, AND  
A. M. ERMANS

The data reported in this monograph provide answers to the two main questions that arose from our previous observations on the role of cassava in the etiology of endemic goitre and cretinism.

*Question 1. What are the nutritional prerequisites for cassava to induce endemic goitre and cretinism in humans and, more specifically, what are the respective roles of iodine and of protein calorie intake in the presence of a cassava-based diet?*

Our study has demonstrated the following points:

- Chronic consumption of large quantities of cassava does not necessarily result in the development of endemic goitre.

- In the presence of a cassava-based diet, the development of goitre is critically related to the balance between the dietary supplies of iodine and SCN.

- In field studies, this balance is most efficiently estimated from the mean value for the urinary I/SCN ratio on casual urine samples

collected in a representative fraction of the total population.

- In normal conditions, the I/SCN ratio is higher than 7. Endemic goitre develops when it reaches a critical threshold of about 3 and becomes hyperendemic, complicated by endemic cretinism, when it is lower than 2. The validity of this ratio as an index of the risk of development of goitre has been demonstrated by comparative studies conducted in different regions of Zaire and at the limit of the hyperendemic goitre area of Ubangi, as well as previous studies in Sicily. The four factors that determine the I/SCN ratio are: the level of iodine intake in the diet; the HCN content of fresh cassava roots and leaves; the efficiency of the detoxification processes used during the preparation of cassava-based foods; and the frequency and quantity of consumption of these foods.

The critical threshold of I/SCN ratio for the development of goitre may be reached either in the presence of a subnormal iodine supply with markedly elevated SCN supply, as in Sicily, or in the presence of severe iodine deficiency and the periodic utilization of poorly detoxified cassava, as in Kivu. When a similar iodine deficiency is associated with a more frequent and a more extreme ingestion of poorly detoxified cassava with a very high content of HCN before detoxification, as in Ubangi, this ratio reaches extremely low values resulting in the development not only of endemic goitre but also of endemic cretinism. When the iodine supply is higher than about 60  $\mu\text{g/day}$ , goitre is not abnormally prevalent even in the presence of a high SCN supply, as reported previously for some parts of Sicily. In such conditions, the I/SCN ratio is indeed higher than the previously mentioned critical threshold. Such a situation probably accounts for the absence of endemic goitre in many populations in the world for which cassava also constitutes a staple food. Finally, massive introduction of cassava because of food shortage in populations previously adapted to iodine deficiency without any abnormal prevalence of goitre results in the development of endemic goitre in these populations.

- There are important differences in the HCN content of cassava-based foods among populations in Zaire for whom cassava is the main staple. These differences may be partly explained by variation in the HCN content of fresh cassava due to genetic or environmental factors, or their interaction, involved in the

biosynthesis of linamarin. They are mainly explained by differences in the traditional detoxification processes. Soaking clearly appears to be the most efficient process. Sun-drying, most commonly used in Ubangi, results mainly in a loss of water from the fresh roots but only partial release of HCN. More efficient detoxification could be achieved by minimal improvement of the traditional detoxification processes used during the preparation of cassava-based foods. Such improvements are cooking cassava flour at a consistently high temperature or for a longer period and using a short period of soaking. However, sustained efforts to introduce these improvements to families in Ubangi, even when they are apparently highly motivated and fully informed, failed completely. This experience stresses the difficulties encountered when trying to modify the food habits of affected rural populations.

- Even extreme protein calorie malnutrition does not appear to greatly impair the endogenous conversion of HCN into SCN in humans. In contrast, protein calorie malnutrition could reduce the development of goitre in affected populations. Consequently, in the presence of severe protein calorie malnutrition, the prevalence of goitre is not an accurate index of the risk of impairment of thyroid function resulting from cassava consumption.

- In adults, a low urinary I/SCN ratio is accompanied by low concentrations of serum thyroid hormones. However, marked hypersecretion of TSH is observed only when the ratio is lower than a critical threshold of about 2. Adaptation of thyroid function to less severe goitrogenic conditions in the environment can take place without marked increase of TSH stimulation. Similar observations have been reported in many endemic goitre areas in the presence of moderate iodine deficiency.

- Infants and children are more sensitive than adults to the antithyroid action of combined iodine deficiency and SCN overload, as they have notably higher TSH than adults in spite of higher concentrations of thyroid hormones, particularly of  $T_4$ .

- Newborns, and to a lesser extent pregnant women, are still more sensitive to the antithyroid action of dietary goitrogens than infants and children. In populations in which the I/SCN ratio is only slightly decreased and thyroid function is unaffected in adults, TSH and  $T_4$  concentrations in cord blood show a clear-cut shift towards high and low values, respectively. When the I/SCN ratio is lower than 2 in the general population, the changes in the

newborn are dramatic and about 10% of them exhibit a caricatural biochemical picture of severe congenital hypothyroidism.

The human placenta is permeable to SCN. The specific role played by SCN during fetal life on the development of hypothyroidism at birth is demonstrated by the comparison of thyroid function in newborns in Kivu and Ubangi where the iodine supply to the mothers is similar but the higher SCN supply in Ubangi is accompanied by markedly higher TSH and lower  $T_4$  in the newborns. In addition, in Ubangi, where we studied two groups of mothers with a similar degree of severe iodine deficiency, the higher SCN supply in one group of mothers was accompanied by a further increase of TSH and decrease of  $T_4$  in the newborns.

Pregnant women and particularly newborns are thus the vulnerable target groups of the population for the toxic action of cassava on thyroid function.

- SCN is significantly lower in breast-fed infants than in newborns, children, and adults because SCN is not concentrated by the mammary gland in humans. Breast feeding thus appears to play a protective role against the development of endemic cretinism, mainly by avoiding a dietary supply of cassava to young infants.

In addition to the information obtained on the goitrogenic action of cassava in human beings, the present studies stressed the importance of two other points related to the problem of endemic goitre.

- Evaluation of the goitrogenic environment in a given area should be based systematically on the simultaneous assessment of the degree of iodine deficiency and of the degree of SCN overload. As a matter of fact, in addition to cassava, a large number of vegetable food-stuffs contain cyanogenic glucosides or thioglucosides that have in common the release of SCN in humans (Podoba and Langer 1964; Van Etten 1969; Langer and Greer 1977). Consequently, the SCN supply should be determined in all endemic goitre areas that have been considered so far solely as a consequence of iodine deficiency.

- The main targets of a goitrogenic environment affecting a population are the pregnant women and, especially, the newborns.

Consequently, in endemic goitre areas, systematic screening for congenital hypothyroid-

ism in the newborn constitutes the most sensitive index for detecting the risk of mental deficiency resulting from environmental goitrogenic factors.

*Question 2. What mechanisms are responsible for mental retardation observed in endemic goitre? Is it caused indirectly by congenital thyroid failure or directly by a toxic action of HCN on the central nervous system?*

The administration of an iodine-deficient diet, alone or when supplemented with HCN or SCN, to pregnant and nursing rats induces thyroid insufficiency in the pups. This insufficiency is accompanied by a striking decrease of the protein, DNA, and cholesterol contents of the cerebellum at weaning, indicating a slowing of cellular growth. Both thyroid failure and alteration of brain composition resulting from HCN-rich diets are prevented when iodine supply is normal.

These data demonstrate that HCN does not affect the process of maturation of the central nervous system in young rats directly but acts indirectly by inducing hypothyroidism after being converted to SCN.

In experimental conditions, huge doses of SCN to pregnant and lactating rats could also result in irreversible cerebral anomalies in the pups, even in the presence of normal iodine supply and thus in the absence of hypothyroidism. However, the doses of SCN used in these experiments were 10–20 times higher than the maximum amounts of SCN obtained by feeding ad libitum rats with fresh cassava roots with a high content of HCN.

Our findings, therefore, suggest that the cerebral abnormalities induced by the consumption of poorly detoxified cassava are mediated by the induction of thyroid failure and, thus, also depend on a critical supply of iodine.



## Chapitre 10

# Résumé et Conclusions Générales

F. DELANGE, P. BOURDOUX ET  
A.M. ERMANS

Les résultats des études décrites dans cette monographie permettent d'apporter des réponses aux deux questions essentielles qui se posaient à la suite de nos travaux antérieurs concernant le rôle du manioc dans l'étiologie du goitre et du crétinisme endémiques :

*Question 1. Quelles sont les conditions nutritionnelles requises chez l'homme pour que le manioc provoque l'apparition de goitre et de crétinisme endémiques, et, plus précisément, quels sont les rôles respectifs de l'apport en iode et en protéines en présence d'une alimentation à base de manioc ?*

Nos études ont démontré les points suivants :

- La consommation chronique d'importantes quantités de manioc ne provoque pas nécessairement l'apparition de goitre endémique.

- En présence d'une alimentation à base de manioc, l'apparition de goitre est liée de manière critique à la balance des apports en iode et en SCN par l'alimentation.

- Au cours d'études sur le terrain, cette balance est appréciée de la manière la plus adéquate par la valeur moyenne obtenue pour le rapport urinaire I/SCN sur des échantillons d'urines uniques collectés parmi une fraction représentative de la population générale.

- Dans les conditions physiologiques, ce rapport est supérieur à 7. Le goitre endémique survient lorsqu'il atteint un seuil critique d'environ 3 et le goitre devient hyperendémique et compliqué par du crétinisme endémique lorsque le rapport est inférieur à 2. La validité de ce rapport en tant qu'index du risque d'apparition de goitre endémique a été démontrée grâce à des études comparatives effectuées dans plusieurs régions du Zaïre ainsi qu'à la limite de la zone hyperendémique de l'Ubangi, et des études conduites antérieurement en Sicile. Les facteurs qui déterminent la valeur du rapport I/SCN sont : l'apport alimentaire en iode, le contenu en HCN des tubercules et des feuilles de manioc cru, l'efficacité des processus de détoxification utilisés dans la préparation d'aliments à base de manioc et la fréquence de consommation de ces aliments et les quantités ingérées.

Le seuil critique du rapport I/SCN qui détermine l'apparition de goitre peut être atteint, soit en présence d'un apport iodé situé à la limite inférieure de la normale et d'un apport en SCN élevé, comme il a été observé en Sicile, soit en présence d'une carence iodée sévère et de la consommation périodique de manioc insuffisamment détoxifié, comme au Kivu. Quand un tel degré de carence iodée va de pair avec une consommation plus fréquente et plus importante de manioc dont le contenu en HCN avant la détoxification est élevé, comme en Ubangi, le rapport I/SCN atteint une valeur extrêmement basse ; il en résulte l'apparition non seulement de goitre mais également de crétinisme endémique. Lorsque l'apport en iode est supérieur à environ 60  $\mu\text{g}$  par jour, il n'existe pas de prévalence anormale de goitre, même lorsque l'apport en SCN est élevé, ainsi qu'il a été montré antérieurement en Sicile. En effet, dans ces conditions, le rapport I/SCN est plus élevé que le taux critique défini ci-dessus. C'est probablement en raison d'une situation similaire qu'il n'existe pas de goitre endémique parmi de nombreuses populations du monde pour lesquelles le manioc constitue également la base de l'alimentation. Enfin, l'introduction massive de manioc en raison d'une pénurie de vivres parmi des populations qui s'étaient jusqu'alors adaptées à une carence iodée sans présenter de taux anormal de prévalence de

goitre, provoque l'apparition de goitre endémique dans ces populations.

- Il existe d'importantes différences en ce qui concerne le contenu en HCN des aliments préparés à partir du manioc dans les populations étudiées au Zaïre et pour lesquelles le manioc constitue l'aliment de base. Ces différences peuvent être expliquées en partie par des différences dans le contenu en HCN du manioc cru en raison de facteurs génétiques ou d'environnement ou leur interaction, impliqués dans la biosynthèse de la linamarine. Ces différences sont cependant dues principalement à des différences dans les procédés de détoxification du manioc utilisés dans ces régions. Le rouissage constitue indiscutablement le procédé le plus efficace. Le séchage au soleil, le plus habituellement utilisé en Ubangi, entraîne une perte en eau des tubercules mais la libération de HCN obtenue par ce procédé est incomplète. Une détoxification beaucoup plus efficace peut être obtenue en introduisant des améliorations minimales dans les processus de détoxification traditionnels utilisés dans cette région, tels qu'une cuisson de la farine de manioc à température constante et plus élevée ou durant une période plus prolongée, ainsi que l'adjonction d'une courte période de rouissage. Cependant, des efforts importants consentis dans le but de persuader certaines familles de l'Ubangi, pourtant très motivées et bien informées, d'utiliser ces améliorations, se sont soldés par un échec complet. Cette observation souligne les difficultés rencontrées lorsque l'on tente de modifier les habitudes alimentaires des populations rurales concernées.

- Une malnutrition protéocalorique même extrême, ne semble pas affecter de manière significative le processus de conversion endogène de HCN en SCN chez l'homme. Par contre, la présence de malnutrition pourrait affecter le processus d'hyperplasie thyroïdienne dans une population soumise à un environnement goitrigène. Par conséquent, en présence d'une malnutrition protéocalorique sévère, l'estimation de la prévalence de goitre pourrait ne pas constituer un indicateur adéquat du risque d'atteinte de la fonction thyroïdienne secondaire à la consommation de manioc.

- Chez l'adulte, un rapport urinaire I/SCN bas va de pair avec une diminution de la concentration sérique des hormones thyroïdiennes. Toutefois, une hypersécrétion importante de TSH n'est observée que lorsque ce rapport est inférieur à un taux critique d'environ 2. Une

adaptation de la fonction thyroïdienne à un environnement goitrigène moins drastique peut survenir sans augmentation importante de la stimulation thyroïdienne par la TSH. Une telle situation a été décrite dans de nombreuses zones d'endémies goitreuses où la carence iodée est modérée.

- Les jeunes enfants sont plus sensibles que les adultes à l'action goitrigène combinée de la carence iodée et de la surcharge en SCN, ainsi que l'atteste l'observation de taux de TSH plus élevés que chez l'adulte en dépit de taux d'hormones thyroïdiennes plus élevés, en particulier de  $T_4$ .

- Les nouveau-nés, et dans une moindre mesure les femmes enceintes, sont encore plus sensibles que les jeunes enfants. En effet, dans des populations où le rapport I/SCN n'est que légèrement abaissé et où la fonction thyroïdienne de l'adulte n'est pas altérée, il existe un déplacement des courbes de distribution des concentrations sériques de TSH et de  $T_4$  sur le sang du cordon vers des valeurs élevées et basses respectivement. Lorsque le rapport I/SCN est inférieur à 2 dans la population générale, les altérations de la fonction thyroïdienne du nouveau-né sont extrêmes et environ 10 % d'entre eux présentent un tableau biochimique d'hypothyroïdie congénitale caricatural.

Le placenta humain est perméable au SCN. Le rôle spécifique joué par le SCN d'origine maternelle durant la vie fœtale dans le déclenchement de l'hypothyroïdie à la naissance a pu être démontré grâce à la comparaison de la fonction thyroïdienne du nouveau-né au Kivu et en Ubangi où l'apport en iode de la mère est identique mais où un apport en SCN plus élevé en Ubangi est accompagné de taux de TSH plus élevés et de  $T_4$  plus bas chez le nouveau-né. De plus, dans deux groupes de mères en Ubangi, soumises au même degré de carence iodée, une surcharge en SCN plus importante dans un groupe que dans l'autre va de pair avec une élévation de la TSH et un abaissement de la  $T_4$  supplémentaires chez le nouveau-né.

Les femmes enceintes et surtout les nouveau-nés constituent donc la fraction cible de la population en ce qui concerne l'action toxique du manioc sur la fonction thyroïdienne.

- Le SCN sérique est plus bas chez le jeune enfant nourri au sein que chez le nouveau-né d'une part et que le grand enfant et l'adulte d'autre part. Cette situation résulte du fait que le SCN n'est pas concentré par la glande mam-



maire dans l'espèce humaine. L'allaitement maternel joue par conséquent un rôle protecteur vis-à-vis du crétinisme endémique, essentiellement en introduisant un délai dans l'administration de manioc au jeune enfant.

Notre travail, en plus des informations qu'il apporte en ce qui concerne l'action goitrigène du manioc chez l'homme, souligne deux points importants relatifs au problème du goitre endémique en général.

- L'appréciation des facteurs goitrigènes d'environnement dans une population devrait comporter à la fois une estimation de l'apport en iode et de la surcharge en SCN. En effet, outre le manioc, de nombreux aliments des régions affectées par le goitre endémique contiennent des glucosides cyanogéniques ou des thioglucosides qui ont en commun la propriété de libérer du SCN dans l'organisme humain (Podoba et Langer 1964; Van Etten 1969; Langer et Greer 1977). Par conséquent, l'apport en SCN devrait être apprécié dans les zones d'endémies goitreuses pour lesquelles le seul facteur étiologique incriminé jusqu'à présent a été la carence iodée.

- Nos études ont montré que les femmes enceintes et surtout les nouveau-nés constituent la fraction cible de la population en ce qui concerne l'action d'un environnement goitrigène.

Par conséquent, le dépistage systématique de l'hypothyroïdie chez le nouveau-né constitue l'index le plus sensible permettant d'apprécier le risque de retard mental résultant de la présence de facteurs goitrigènes dans l'environnement.

*Question 2. Quels sont les mécanismes responsables du retard mental observé dans le goitre endémique ? S'agit-il d'une insuffisance thyroïdienne congénitale ou de l'action toxique directe du HCN sur le système nerveux central ?*

L'administration d'une diète carencée en iode, seule ou en présence de suppléments de HCN ou de SCN à des rates gravides et allaitantes provoque une hypothyroïdie chez les rats. Cette hypothyroïdie est accompagnée d'une diminution importante du contenu du cervelet en protéines, DNA et cholestérol au moment du sevrage, indiquant un ralentissement du processus de croissance cellulaire. A la fois l'hypothyroïdie et les altérations cérébrales provoquées par le HCN sont évitées lorsque l'apport en iode est normal.

Ces observations démontrent que le HCN n'affecte pas par lui-même le processus de maturation du système nerveux central chez le jeune rat et que les altérations observées résultent de l'hypothyroïdie induite par le SCN provenant de la conversion endogène du HCN.

Dans des conditions expérimentales, l'administration à des rates gravides et allaitantes de quantités de SCN extrêmement élevées provoque des lésions cérébrales irréversibles chez le raton, même en présence d'un apport iodé normal et donc en l'absence d'hypothyroïdie. Cependant, les quantités de SCN sont 10 à 20 fois plus élevées que celles atteintes lorsque des rats sont nourris ad libitum avec du manioc cru riche en HCN.

Nos résultats suggèrent par conséquent que les anomalies cérébrales résultant de la consommation de manioc mal détoxifié sont la conséquence de l'hypothyroïdie induite par le SCN qu'il libère et dépendent donc de manière critique de l'apport iodé.



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## Errata

*Role of Cassava in the Etiology of Endemic Goitre and Cretinism*. IDRC-136e. Editors: A.M. Ermans, N.M. Mbulamoko, F. Delange, and R. Ahluwalia. Ottawa, IDRC, 1980. 182 p.

### Pages

- 78 Fig. 43, Upper panel. For  $r = 0.741$  Read  $r = -0.741$
- 117 Fig. 66. For  $r = 0.71^{***}$  Read  $r = -0.71^{***}$
- 122 Table 53, TSH ( $\mu\text{U/ml}$ ), Pregnant women in Zaire. For  $1.53 \pm 2.1$  (221) Read  $15.3 \pm 2.1$  (221)
- 123 Column 1, Para. 3, Line 3. For ( $r = 0.130, P < 0.1$ ) Read ( $r = 0.130, P > 0.1$ )
- 141 Fig. 76. For  $r = 0.61$  Read  $r = -0.61$
- 147 Column 2, Para. 1, Line 1. For 3 to 42% Read 3 to 70%

